ICDDRB: Centre for Health & Population Research RRC APPLICATION FORM

RESEARCH PROTOCOL

FOR OFFICE USE ONLY

Protocol No: 2000-010 Date received: 07.03.2000

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

Project Title: A double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of Racecadotril in the treatment of acute diarrhoea resulting from *Vibrio cholerae* in adults.

Theme and key words: Clinical response of Hidrasec (anti-entephalinase) in patients infected with *V. cholerae*. Hidrasec, *V. Cholerae*, Severe dehydration, Adult patients

Principal Investigator: Dr. Nur Haque Alam Division: Clinical Sciences Division Phone: 880-2-8711751-60/2311

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Co-Principal Investigator(s): Dr. George Fuchs

Co-Investigator(s): Dr. Wasif Ali Khan
Dr. Hasan Ashraf

Student Investigator/Intern: Not Applicable

Collaborating Institute(s): Not Applicable

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- ☑ Male
- ☑ Females

Age
- ☐ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☐ Others (specify Age **15 - 55 years**)

Project / study Site *(Check all the apply)*:
- ☑ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: _____
- ☐ Multi centre trial
  (Name other countries involved)

Type of Study *(Check all that apply)*:
- ☐ Case Control study
- ☐ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☑ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ☐ Others

Targeted Population *(Check all that apply)*:

- [x] No ethnic selection (Bangladeshi)
- [ ] Bangalee
- [ ] Tribal groups
- [ ] Expatriates
- [ ] Immigrants
- [ ] Refugee

Consent Process *(Check all that apply)*:

- [x] Written
- [ ] Oral
- [ ] None
- [x] Bengali language
- [ ] English language

Proposed Sample size: Total sample size: 110 ☐

Sub-group _____ ☐ _____ ☐

_____ ☐ _____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- [ ] Human exposure to radioactive agents?
- [ ] Fetal tissue or abortus?
- [ ] Investigational new device? (specify_____)
- [ ] Existing data available from Co-investigator
- [ ] Human exposure to infectious agents?
- [ ] Investigational new drug
- [ ] Existing data available via public archives/source
- [ ] Pathological or diagnostic clinical specimen only
- [ ] Observation of public behavior
- [x] New treatment regime

Yes/No

- [ ] [x] Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?
- [ ] [x] Does the research deal with sensitive aspects of the subject's behavior; sexual behavior, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

- [ ] [x] a. place the subject at risk of criminal or civil liability?
- [ ] [x] b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- [ ] greater than minimal risk
- [ ] no risk
- [x] no more than minimal risk
- [ ] only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

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Yes/No

☐ ☐ Is the proposal funded?

If yes, sponsor Name: SMITHKLINE BEECHAM Pharmaceuticals, UK

Is the proposal being submitted for funding? Not Applicable

☐ ☐ If yes, name of funding agency: Not Applicable

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director. Not Applicable

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**
(Day, Month, Year - DD/MM/YY)	*a.* ***Ist Year*** ***2nd Year*** ***3rd Year*** ***Other years***
Beginning date 01 May 2000	
End date 31 July 2001	*b.* ***Direct Cost :*** $85,286 ***Total Cost :*** $106,608

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Dr. George Fuchs
Name of the Division Director

Signature

7.3.2000
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the re-

Signature of PI

Date: 6-3-2000

Name of Contact Person (if applicable)

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SB
SmithKline Beecham
Pharmaceuticals

International Medical Department

CONFIDENTIAL

52607/RACECADOTRIL (HIDRASEC™)

A double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of Racecadotril in the treatment of acute diarrhoea resulting from *Vibrio cholerae* in adults.

Protocol number 52607/002

Final protocol 28 February 2000

SB
SmithKline Beecham

Pharmaceuticals

Clinical Unit : Tropical Medicines, International Medical Department.

Drug Name : Racecadotril , Hidrasec ™

Compound No : SB 52607

Protocol Title: A double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of Racecadotril in the treatment of acute diarrhoea resulting from Vibrio cholerae in adults.

CPMS No : 52607 / 002

Principal Investigator Dr Nur Haque Alam
Co Principle Investigator Dr George Fuchs
Co Investigator Dr Wasif Ali Khan
Co Investigator Dr Hasan Ashraf

Authors:

Dr Nur Haque Alam
Dr George Fuchs
Sally Coulden
Dr. Palma Seljan

SB Site Monitor :

I, the undersigned, have reviewed this protocol, including Appendices and I will conduct the study as described and will adhere to the Ethical and Regulatory Considerations stated. I have read and understood the Investigator Brochure.

Investigator ..... **Date** 6-3-2000

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List of Appendices

SYNOPSIS

Compound / Protocol No. **SB – 52607 / 002**

Indication

Patients suffering with life threatening diarrhoea due to *Vibrio cholerae* infection, suitable to be treated with a novel oral antisecretory agent in addition to standard treatment.

Protocol Title

A double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of Racecadotril in the treatment of acute diarrhoea resulting from Vibrio cholerae in adults.

Rationale

Cholera represents the archetypal disease in the context of small-intestine secretory (watery) diarrhoea.[1,2,3] Cholera is endemic in India, Pakistan, Bangladesh, Afghanistan and many other countries in south-east Asia. In recent years, epidemics have occurred in the Middle-East, South America and Africa;[2] most have been localised, although from time to time global pandemics, associated with new strains can occur. Outbreaks involving air travellers have now been recorded. It tends to affect young people more often than the elderly. If untreated, the disease has a 20 - 80% mortality. Death results from dehydration, vascular collapse and renal failure.

Cholera is a tropical disease, the organism, *V cholerae,* never actually entering any tissue of the body. It is swallowed either with water or food. Cholera may be present as an almost asymptomatic state, as mild diarrhoea, or as the typical 'full-blown' syndrome. In its extreme manifestation, cholera is one of the most rapidly fatal illnesses known. A healthy person may become hypotensive within few hours of diarrhoeal onset and death can occur from cholera as fast as within 12 hours of diarrhoeal onset.

There has been considerable enthusiasm for the development of antisecretory drugs which would, through an understanding of the cellular mechanisms of the secretory processes, turn off intestinal secretion in cholera patients. Such an agent might simplify the treatment of cholera patients since it would further shorten the duration of the illness and decrease the course of rehydration needed. Several drugs have been shown to have an antisecretory effect in experimental animal studies, and some of these have been tested in patients. Chlorpromazine, a phenothiazine with antisecretory properties in animal models, also proved to significantly reduce the stool volume of cholera patients if they were rehydrated and maintained on intravenous fluids and were not given antibiotics.[9,10] Unfortunately, patients taking chlorpromazine also developed somnolence which interfered with the administration of oral rehydration fluid; hence, chlorpromazine is not recommended.

Although antisecretory drugs could potentially assist in the treatment of cholera patients, none so far have proved to be of use in routine clinical management.

Clinical studies have demonstrated, both against placebo and loperamide, the efficacy of Racecadotril in acute diarrhoea[28]. Its antidiarrhoeal effect seems to result primarily from an antisecretory mechanism. An antisecretory or antidiarrhoeal effect has already been demonstrated in dogs with cholera toxin-induced secretion[24], administration in rats after cholera toxin and in man after cholera toxin administration.[26] The latter showed that Racecadotril abolished CT-induced secretion in the human jejunum.

The objective of this study is to assess whether Racecadotril through its antisecretory effect would be useful when added to the standard treatment of cholera including oral rehydration solution. Secondary objectives are to determine whether it has any influence on other aspects of the disease such as vomiting and duration of diarrhoea. Previous studies have shown that the success of oral therapy in cholera is limited by high purging rate and by vomiting.[27] It is hoped that in severe cases the need to use i.v. hydration might be reduced, thus allowing an earlier introduction of oral rehydration.

Objectives(s)

To assess the efficacy of Racecadotril in the treatment of acute diarrhoea due to *V. cholerae*

To assess the safety profile (adverse experiences,laboratory safety parameters and concomitant medication) in patients treated with Racecadotril.

Study Population

110 patients presenting with acute diarrhoea due to *V. cholerae* will be enrolled at the International Centre for Diarrhoeal Disease Research Dhaka, Bangladesh. This centre treats more than 100,000 diarrhoeal patients each year. This is a single centre study.

Study Design

This is a double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of oral Racecadotril for the treatment of acute diarrhoea resulting from *Vibrio cholerae* in adults.

Patients who fulfil the study entry criteria and have given written, dated, informed consent will receive either Racecadotril 100 mg 6 times daily po, or placebo, this treatment will be given as an adjunct to standard fluid replacement therapy for a maximum of 3 days. All patients will be admitted to hospital for the course of the study.

Efficacy Parameters

Primary variable:

- Total stool output, in g/kg of body weight for the duration of diarrhoea or the end of study medication (3 days) whichever is the quicker.

Secondary variables:

- Total stool output in g/kg at 24 and 48 hours time periods.
- Intake of ORS and plain water, in ml/kg of body weight, from initiation of treatment until the the resolution of diarrhoea or the end of study medication (3 days).
- Weight gain, expressed as percent increase in weight, from initiation of study treatment until the resolution of diarrhoea or the end of study medication (3 days).
- Occurrence of hypernatraemia or hyponatraemia

SAFETY PARAMETERS:

The following will be evaluated:
Adverse experiences, laboratory safety paramenters and concomitant medication. To note that this compound 'Racecadotril' is currently licensed for use in patients with acute watery diarrhoea it is nevertheless of paramount importance to continue to collect all safety data.

1.0 INTRODUCTION

Cholera represents the archetypal disease in the context of small-intestine secretory (watery) diarrhoea."[23] Cholera is endemic in India, Pakistan, Bangladesh, Afghanistan and many other countries of South-East Asia. In recent years, epidemics have occurred in the Middle East, South America and Africa;[2] most have been localised, although from time to time global pandemics, associated with new strains can occur. Outbreaks involving air travellers have now been recorded. It tends to affect young people more often than the elderly. If untreated, the disease has a 20-80% mortality. Death results from dehydration, vascular collapse and renal failure.

Cholera is a tropical disease, the organism, *V. cholerae,* never actually entering any tissue of the body. It is swallowed either with water or food. Cholera may be present in an asymptomatic state, as mild diarrhoea, or as the typical 'full-blown' syndrome. In its extreme manifestation, cholera is one of the most rapidly fatal illnesses known. A healthy person may become hypotensive within few hours of the onset of symptoms and may die as quick as 12 hours from the onset of diarrhoeal illness if no treatment is provided.

There are no prodromal symptoms. The incubation period varies from 18 to 48 hours. Onset is sudden, and mild diarrhoea rapidly gives way to the passage of a large volume of opalescent fluid - the classic 'rice-water' stools. Up to 30 litres of fluid, containing a high concentration of *Vibrio* spp. organisms, may be passed in 24 hours.[3] All the symptoms and signs of cholera derive from the rapid depletion of water and salts from the intravascular and extracellular spaces of the body by loss into the gut lumen. Vomiting is often present at the early stages of cholera and assumes particular importance with respect to the oral replacement of fluid losses. Acidosis is commonly observed in severely dehydrated cholera patients.

The main, though probably not exclusive, site of action for cholera toxin is the small intestine and especially the duodenum and jejunum.[4-5] This site reflects the large number of cholera vibrios and, thus, the comparatively large amounts of cholera toxin released there, as well as a decreasing secretory response to cholera toxin from the proximal towards the distal part of the intestine.[6] Cholera is regarded as the archetypal c-AMP-mediated intestinal secretory process. The precise biochemical mechanisms by which cholera toxin and c-AMP inhibit active NaCl absorption and cause active chloride and bicarbonate secretion are less well known. In addition to fluid losses it is very likely that the cholera toxin triggers a variety of other phenomena. Because the intestine is a very complex endocrine organ and many hormones are governed by the levels of cyclic nucleotides in the tissues that secrete them, it is likely that cholera toxin disorganises the delicately integrated humoral and neural systems that co-ordinate normal intestinal function in ways that have not as yet been defined. Lundgren and co-workers have proposed an important role for the enteric nervous system in the pathophysiology of enterotoxin-induced secretory states including cholera.[7] Clearly, further work is required to define the role of nerves and neurotransmitters in cholera secretion, an area of research that might also give new clues to pharmacological intervention of secretory diarrhoeas.[8]

There has been considerable enthusiasm for the development of antisecretory drugs which would, through an understanding of the cellular mechanisms of the secretory processes, turn off intestinal secretion in cholera patients. Such an effective antisecretory agent might simplify the treatment of the cholera patients since it would further shorten the duration of the illness and decrease the course of rehydration needed. Several drugs have been shown to have an antisecretory effect in experimental animal studies, and some of these have been tested in patients. Chlorpromazine, a phenothiazine with antisecretory properties in animal models, also proved to significantly reduce the stool volume of cholera patients if they were rehydrated with and maintained on intravenous fluids and were not given antibiotics.[9;10] A follow-up study carried out to determine if this observation could be confirmed when chlorpromazine was added to a routine treatment regimen, including antibiotics and ORT showed no overall difference between treatment groups; however, there was a suggestion that chlorpromazine did have some antisecretory effect in severely purging children.[11] Unfortunately patients taking chlorpromazine also developed somnolence which interfered with the administration of oral rehydration fluid; hence, chlorpromazine is not recommended. If another drug could be identified which shows an antisecretory activity without producing somnolence, it might still be useful. Other drugs have been tested in patients[12;13;14-1] ; however, they showed either minimal or no benefit. In summary, although antisecretory drugs could potentially assist in the treatment of cholera patients, none so far have proved to be of use in routine clinical management.

The enkephalins, the endogenous opiate substances first discovered in *1975,* play an important physiological role acting as neurotransmitters most notably along the digestive tract. After their release and the stimulation of opiate receptors, the enkephalins are rapidly inactivated by enkephalinase. It was assumed that preventing this degradation would provide a therapeutic means to protect endogenous enkephalins and thereby reinforce their physiological actions. The discovery and characterisation of enkephalinase in brain and the demonstration that this peptidase was responsible for the inactivation of endogenous enkephalins paved the way for the novel therapeutic approach.

Enkephalinase is present all along the gastrointestinal tract. Enkephalins are endogenous opioids which elicit intestinal antisecretory activity without affecting intestinal transit or motility. Tiorfan, by the inhibition of enkephalinase reinforces the physiological activity of endogenous erikephalins and shows intestinal antisecretory [16-18] activity without affecting intestinal transit. This approach is novel: although other antidiarrhoeals also act on the enkephalin receptor pathway, they act by mimicking endogenous enkephalins at both lambda and mu receptors. This approach results in decreased motility and intestinal transit, and causes the side effects of drugs such as loperamide. In diarrhoeal disease states the lambda receptor is upregulated, and thus reduction of erikephalin breakdown by inhibition of enkephalinases will promote lambda-type activity at the expenses of mu activity. This will lead to a reduction of salt/water excretion without any transit effects.

Pharmacokinetic profiles of Racecadotril were studied using the level of plasma enkephalinase activity as a measure. After a single oral administration of Racecadotril™, the maximal inhibition **(Cmax)** of enkephalinase was 80%. The peak inhibition **(Tmax)** is observed between 1 and 2 hours after the administration of the drug. The biological half-life was three hours. With 300 mg, enkephalinase is significantly inhibited for up to 8 hours. Kinetic parameters are identical after a single and after multiple administration.

Studies of pharmacodependence and tolerance (rat, monkey) demonstrated the absence of addictive properties of Racecadotril. Racecadotril is distributed in peripheral organs and does not cross the blood barrier after oral administration. Pharmacological studies in animals have demonstrated the activity of racecadotril on several experimental models of diarrhoea. The intestinal antisecretory effect is observed only in case of hypersecretion and not in the basal state, and is inhibited by naloxone demonstrating the enkephalin/enkephalinase pathway of racecadotril activity.[16]

Clinical studies demonstrated, both against placebo and loperamide, the efficacy of Racecadotril in acute diarrhoea. The efficacy of Racecadotril has been demonstrated in three double-blind studies against placebo[19-21] and in two double-blind studies against loperamide.[22,23] Its antidiarrhoeal effect seems to result primarily from an antisecretory mechanism. This may explain both the more rapid resolution of symptoms related to fluid accumulation in a distended bowel and the much lower occurrence of constipation in Racecadotril - treated patients. In addition to the improved patient comfort (and social cost) that can be expected from the use of antidiarrhoeal agents acting via a purely antisecretory mechanism, these drugs are likely to prevent several side effects associated with the use of antimotility drugs. These include facilitation of bacterial colonisation, invasion of *Shigella,* extension of the period of excretion of bacterial pathogens, and precipitation of ileus and bowel dilatation ('toxic megacolon')[21 23]

An antisecretory or antidiarrhoeal effect has already been demonstrated in dogs with cholera toxin-induced secretion[24,] [L Bueno, personal communication], in rats after cholera toxin administration[25] and in man after cholera toxin administration.[26] The latter showed that Racecadotril abolished CT-induced secretion in the human jejunum.

The objective of this study is to assess whether Racecadotril through its antisecretory effect proves effective in the treatment of acute diarrhoea due to *V. cholerae*. Secondary objectives are to compare stool output and fluid input at various intervals during the study. I addition weight gain, safety and tolerability of the study medication will be monitored. Previous studies have shown that the success of oral therapy in cholera is limited by high purging rate and by vomiting.[27] It is hoped that in severe cases the need to use i.v. hydration might be reduced, thus allowing an earlier introduction of oral rehydration.

Side effects as reported from clinical studies in association with Hidrasec includes; GI tract- Nausea/vomiting, epigastric pain, constipation, abdominal bloating; CNS - Dizziness, asthenia, headache, mood change, insomnia; Skin - Skin rash, pruritus, acne; Uro-nephrology - Increase or decrease of urinary frequencies.

For more information on the safety of the product please refer to the Investigator Brochure.

2.0 OBJECTIVES

2.1 Primary

The expectation is that a drug reducing the rate of fluid loss in cholera will decrease the need for intravenous fluid permitting a wider range of usefulness for oral rehydration. The main objective of this study is to assess whether or not Hidrasec™ will be beneficial when added to the standard treatment for cholera.

The primary objective is to assess the total stool output in g/kg bodyweight from the time of randomisation until either the resolution of diarrhoea, or the end of study medication (3 days), which ever is the sooner.

2.2 Secondary

- To compare stool output in g/kg at 24 hours and 48 hours of treatment.
- To compare the intake of ORS and plain water, in ml/kg of body weight, from initiation of treatment with the study drug until the resolution of diarrhoea or the end of study medication (3 days).
- To compare weight gain, expressed as percent increase in weight, from initiation of study treatment until the resolution of diarrhoea or the end of study medication (3 days).
- To compare the occurrence of hypernatraemia or hyponatraemia.
- Safety and tolerability of the study medication (adverse experiences, laboratory safety parameters and concomitant medication).

3.0 STUDY DESIGN OVERVIEW

This is a double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of oral Racecadotril for the treatment of acute diarrhoea resulting from *Vibrio cholerae* in adults.
Patients who fulfil the study entry criteria and have given written, dated informed consent will be randomised to either:

- Racecadotril 100 mg 6 times daily po (4hourly)
- Placebo 6 times daily po (4hourly)

This treatment will be given as an adjunct to standard fluid replacement therapy for a maximum of 3 days. All patients will be given one standard dose of doxycycline 300mg once randomised onto the study and all patients will admitted to hospital for the course of the study.

Patients will participate in a 4 – 6 hour screening period prior to being randomised into the study. During this period they will undergo rehydration therapy in the form of intra venous therapy and oral fluid only. The patient will then be reassessed at the end of the screening period, providing they have been adequately rehydrated, sufficient not to require further IV therapy the patient can be randomised into the study. Once randomised they will be observed closely and all observations will be documented in the CRF up until 24 hours following resolution of their diarrhoea, (defined as 12 hours with no stools or 2 consecutive normal stools), or until the patient is discharged from the study.

4.0 STUDY POPULATION

4.1 Number of subjects

110 clinically evaluable patients, aged ≥15 years to ≤55 years inclusive, presenting with acute diarrhoea due to V *cholerae* having started less than 24 hours prior to being enrolled at the International Centre for Diarrhoeal Disease Research in Dhaka.

4.2 Inclusion Criteria

Patients may be included into the screening of this study if the following criteria are satisfied:

1. Patients aged ≥15 years to ≤55 years.
2. A clinical history of acute watery diarrhoea without faecal blood or mucus of less than 24 hours duration.
3. Clinical signs and symptoms of severe dehydration entering screening. (refer to Appendix E)
4. Written informed consent has been obtained.

4.3 Exclusion Criteria

Patients should be excluded from the screening of this study if any of the following criteria apply:

1. The patient has demonstrated a previous hypersensitivity reaction to enkephalinase inhibitors and/or mu receptor agonists.
2. The patient has received antimicrobial or antidiarrhoeal medication within the seven days prior to admission. Eg. Kaolin, Pectin, Cholestyramine and opioid classes of

drugs (loperamide, lomotil etc.)

3. The patient has chronic or iatrogenic diarrhoea or dysenteric syndrome characterised by faecal blood loss.

4. The patient has acute ulcerative colitis or pseudomembranous colitis associated with broad-spectrum antibiotics.

5. Clinical signs and symptoms of no signs or moderate dehydration on entering screening. (refer to Appendix E)

6. The patient has received an investigational drug within 6 months of the screening visit or is scheduled to receive such a drug during the study period.

7. The patient has a concomitant infection or any other underlying disease which would compromise the diagnosis and the evaluation of the response to the study medication.

8. The patient has a known clinical history of renal dysfunction or hepatic dysfunction.

9. Patient that is unwilling or unable to take part in this study or refuses to sign informed consent.

10. Women whose LMP > one month before enrollment must be excluded.

11. The patient has previously been enrolled in the study.

5.0 CONDUCT OF THE STUDY

5.1 Ethics and Regulatory Considerations

The study will be conducted according to Good Clinical Practice and the Declaration of Helsinki (Protocol Appendix A) and relevant national guidelines.

5.1.1 Ethics Review Committee/Institutional Review Board (ERC/IRB)

ERC/IRBs must be constituted according to the local laws/customs of the participating country.

This protocol will be submitted to an appropriate Committee or Board and their written unconditional approval obtained and submitted to the sponsor before commencement of the study. SB will supply relevant data for the investigator to submit to the hospital /university/independent ERC/IRB for the protocol's review and approval. Verification of the ERC/IRB unconditional approval of the protocol and either the written informed consent statement or sample oral witnessed consent form with written information to be given to the subjects will be transmitted to the SB Study Monitor prior to shipment of drug supplies and CRFs to the site. This approval must refer to the study by exact protocol title and number, identify the documents reviewed and state the date of review.

The ERC/IRB must be informed by the investigator of all subsequent protocol amendments and of serious or unexpected adverse experiences occurring during the study which are likely to affect the safety of the subjects or the conduct of the study. Approval of such changes must be transmitted in writing to the SB Study Monitor via the investigator.

5.1.2 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki (Protocol Appendix A) should be implemented in each clinical study before protocol-specified procedures are carried out.

Information should be given in both oral and written form whenever possible and deemed appropriate by the ERC/IRB. Subjects, their relatives, guardians or, if necessary, legal representatives must be given ample opportunity to inquire about details of the study.

The consent form generated by the investigator with the assistance of SB, must be approved (along with the protocol) by the ERC/IRB and be acceptable by SB. Consent forms must be in a language fully comprehensible to the prospective subject. Informed consent shall be documented by the use of a written consent form approved by the ERB/IRB and signed by the subject or the subject's legally authorised representative.

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations. This form may be read to the subject or the subject's legally authorised representative, but, in any event, the investigator shall give the either the subject or the representative adequate opportunity to read it before it is signed.

Consent must be documented either by the dated signature of the subject or by the subject's legally authorised representative. The signature confirms the consent is based on information that has been understood. Each subject's signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or SB professional and Regulatory Compliance persons.

In countries where written informed consent contravenes local custom, informed consent may be gained orally. Full and comprehensive information must be communicated to the potential subject (or his legal representative) in the presence of a witness. The witness will be an independent third party, i.e., not a nominated co-investigator. The witness will sign the informed consent form (testifying that informed consent has been given orally) along with the investigator (or his/her nominated representative).

5.2 General Instructions

A case report form (CRF) should be completed for each patient entered into the study.

Patients will receive study medication under supervision of the study physician or nurse. At the end of the study, the investigator, assisted by the SB Clinical Research Scientist, will return the used and unused medication together with a completed drug accountability form.

5.3 Study Flow

See Appendix F for the outline of study flow.

5.3.1 Screening (Day 1, Hour –6 / -4 to 0 maximum of 6 hours rehydration)

The screening visit will consist of the following assessments , all information will be recorded in the Case Report Form.

- The patient will be checked for compliance with the Inclusion/Exclusion criteria
- Informed written consent will be obtained from the patient/ legal guardian by the investigating physician before study specific procedures are undertaken before entry into the study.

- A patient demography, comprising of age and race.

- Evaluation of vital signs (supine pulse, blood pressure and weight) will be performed and where possible the same person should take these measurements on each occasion during the study.

- A medical/surgical history will be taken as well as performing a full physical examination.

- Documentation of any baseline signs or symptoms eg. episodes of vomiting.

- Duration of diarrhoea prior to inclusion into the study and the number of stools in the preceding 24 hours will be recorded.

- Assessment of dehydration category as defined in section *5.4.1.*

- Documentation of prior or concomitant medication

- All patients entering this study will be rehydrated with an intravenous solution to replace the fluid deficit during the first four to six hour observation period, as defined in section 5.4.2. At 0 hours (after a minimum of 4 hours and a maximum of 6 hours IV rehydration) the investigator will form a clinical opinion based on all the information to date whether the patient is:

  - Adequately rehydrated
  - Has completed and discontinued IV therapy
  - Is able to maintain adequate hydration with oral fluids alone.

  If the patient is considered moderately or severely dehydrated by the investigator at this point and requires further IV rehydration he should be excluded from the study.

- Patients during this screening period should only drink water and ORS. They should not be given food.

- A blood sample will be obtained to measure packed cell volume, serum specific gravity, serum sodium, potassium, chloride and bicarbonate concentrations. If abnormalities are found, these will be recorded in the Case Record Form and followed up daily until complete resolution or until the results are no longer considered clinically significant.

- A stool sample will be obtained for examination by dark field microscopy.

5.3.2 Admission to the study (Day 1, Hour 0)

At this visit the following assessments/procedures will be carried out :

- Assessment of vomiting in the four to six hours previous. This will be measured as number of episodes.
- Record vital signs and weight.
- Assessment of dehydration (5.4.1) & rehydration (5.4.2)
- Assessment of stool output and rate of stool output.
- Assessment of volume of i.v. fluid infused, and oral fluids injested.
- Assessment of urine output
- Assessment of concomitant medication
- Assessment of eligibility for inclusion into the study.
- Randomisation and administration of study medication.

Patients should only be permitted to be randomised into the study if they fit the following criteria:

Inclusion:

- **Purging rate ≥ 5 ml/kg/hr body weight during the four to six hour basal period.**
- **Dark-field microscopic indicates evidence of V *cholerae* in the stool.**
- **IV therapy has been discontinued**
- **Patient is able to maintain adequate hydration with oral fluids alone**

Exclusion:

- **Purging rate < 5 ml/kg/hr body weight during the four hour basal period.**
- **Clinical signs and symptoms of dehydration.**
- **IV therapy required**

5.3.3 Patient randomisation

All patients who meet the entry criteria will be randomised to either:

Group A
Patients will receive Racecadotril 100 mg (1 capsule) 6 times a day (every 4 hours)

Group B
Patients will receive placebo (1 capsule) 6 times a day (every 4 hours)

A randomisation code will be generated by the data management company. The treatment allocation will be balanced, with equal numbers of patients being allocated each study medication. Each eligible patient will be assigned the next patient number available at the centre and thereby will be assigned randomly to one of the two

treatment arms. (see 6.3 for blinding)

In addition, all patients will receive one doxycycline 300mg capsule immediately after randomisation, if the patient vomits within 10 minutes of administration they should be redosed. If the patient vomits on 2 consecutive occasions they must be withdrawn from the study. (see 6.2 for redosing)

All treatment groups will receive oral rehydration therapy as standard treatment. Treatment will be continued until resolution, (defined as 12 hours with no loose/ watery stools or as the output of two normal stools) or treatment for a maximum of 3 days.

Diet:
All patients will receive the standard hospital diet, three times a day.
Breakfast (7am) – bread, sugar and banana;
Lunch (12.30pm) – rice, lentils and fish/meat.
Supper (7pm) rice, lentil /vegetables and meat or fish.

5.3.4 On-Therapy Evaluations

Patients will be observed by study staff continuously, observations will be recorded four hourly until 24 hours following resolution of diarrhoea, (defined as 12 hours with no stools or 2 consecutive normal stools), or until the patient is discharged from the study. All patients will have a maximum of 3 days treatment and will then be assessed in hospital for a further 24 hours after the end of treatment.

Assessments:

- Assessment of stool output and rate of stool output.
- Assessment of volume of ORS/water intake & (IV infused as appropriate).
- Assessment of urine output
- Assessment of vomiting
- Assessment of rehydration
- Assessment of dehydration
- Assessment of clinical vital signs and weight.
- Collection of information on concomitant medication
- Collection of information on adverse experiences

5.3.5 End of Therapy

Patients will remain in the hospital for a minimum of 24 hours after diarrhoea resolution, (12 hours with no stools or 2 consecutive normal stools) or a maximum of 3 days treatment followed by 24 hour observation, observations will continue 4 hourly until discharge.

5.3.6 Study Conclusion

The following assessment will be performed at study conclusion

- Collection of information on concomitant medication
- Assessment of vomiting
- Assessment of rehydration
- Assessment of weight and vital signs
- Assessment of stool output
- Assessment of dehydration
- Assessment of IV infused (if appropriate)
- Assessment of water/ ORS intake
- Assessment of urine output
- Collection of stool sample
- Collection of blood sample
- Study medication compliance
- .Review of any adverse experiences
- Did the patient complete the study as planned
- Evaluation of clinical response (see section 5.4.6)
- Date of last dose of study medication
- Time of last dose
- Date of discharge

5.3.7 Follow-up Evaluation

Those patients who were withdrawn from the study prior to the end of therapy will require a follow up safety assessment (not an efficacy assessment) at 28 days after the last dose of study medication. Adverse events and concomitant medication(s) will be recorded and assessed at this visit.

If the patient was not withdrawn but completed the treatment period, they are not required to attend this visit.

5.4 Details of study procedures

5.4.1 Dehydration Category

On admission to the hospital and once considered suitable for screening, the patient will be placed on a cholera cot, weighed and their clinical signs recorded to permit a correct assessment of dehydration. The patient will then be categorised into one of the three dehydration categories: mild, moderate or severe. These categories correspond approximately to 5%, 7.5% and 10% dehydration, respectively. (fluid

deficit relative to body weight after hydration). Only patients that present with severe dehydration at screening will be entered in to the 4-6 hour screening period. Patients will then be reassessed at the end of the screening period. Only patients that present at this stage with mild dehydration will be randomised into the study. Patients with moderate or severe dehydration will not be randomised.

5.4.2 Rehydration Procedures

During the first four to six hour observation period (-6 /-4 to 0 hours), patients will be rehydrated with an intravenous solution containing sodium 133 mmol/l, potassium 13 mmol/l, bicarbonate equivalent 48 mmol/l and chloride 98 mmol/l to replace the fluid deficit. The volume of i.v. fluid given should be 10% of the body weight, ongoing stool losses will be replaced with the same solution. Patients will also be encouraged to drink oral rehydration salts (ORS) and water during this period.
After the four- six hour observation period, consenting patients presenting with mild dehydration and with a purging rate $\geq$ 5 ml/kg/hr body weight during that period will then be admitted into the study and maintained with a glucose oral rehydration solution to match the stool output. Patients that require further IV therapy should not be entered into the study.(Clarification: Moderately or severely dehydrated patients should not be randomised into the study)

The progress of rehydration will be assessed by determining the changes in the body weight, blood pressure, heart rate and skin turgor along with intake of rehydration fluid and stool output. This will be recorded every four hours after admission until discharge. Intake and output measurements of fluids including urine output will be measured to the nearest 5 ml. Intake of ORS and water will be recorded accurately, from admission to hospital until hospital discharge.

5.4.3 Assessment of Diarrhoea

Duration of diarrhoea is defined as:
The time (in hours) from randomisation and initiation of study medication to diarrhoea resolution: Time of last abnormal (loose or watery) stool. This is then followed by12 hours with no stools or 2 consecutive normal stools.

Total stool output will be measured and expressed as g/kg of body weight (at baseline), this will be measured from:
Time of randomisation /initiation of study medication, to diarrhoea resolution or time of discharge, whichever is the sooner.

The stool output, in g/kg, will be measured for every 24 hours of treatment with the study medication. The patients weight based on weight at 0 hours (randomisation).

Definition of stool consistency:

Watery/Liquid stool : Stool that can be poured from one container to another.
Soft stool : Stool that takes the shape of the container where it is poured.
Formed stool : Stool that remains in its original shaped wherever it is poured.

5.4.4 Laboratory Evaluations

The following parameters will be measured after the IV rehydration period but before randomisation into the study. They will then be repeated 24 hours later and on the day of discharge if the initial tests are considered by the Investigator to be clinically normal. If the values are found to be out of range and clinically abnormal for that patient at any point during the course of the study they must then be repeated daily until the values return to a clinically normal state.

Haematology and Clinical Chemistry

The following will be evaluated:

- Haematocrit, and white blood cell count.
- Specific gravity, BUN, creatinine, sodium, potassium, chloride and bicarbonate..

5.4.5 Stool Examination

On admission, stool samples will be obtained for microscopic (dark field) examination to confirm the presence of *vibrios*. To determine other possible bacterial causes of diarrhoea, rectal swabs will be obtained and cultured for ***V cholerae, Shigella, Salmonella*** and ***Camphylobacter*** species. A stool culture and colony count will be performed daily for cholera up to the time of discharge. (A repeat culture will be taken 24 hours after the resolution of diarrhoea.)

5.4.6 Assessment of Response at the End of Therapy

All patients will be observed closely up until resolution of diarrhoea. (12 hours with no stools or 2 consecutive normal stools). Clinical response to the study drug will be assessed as follows:

Clinical success

Defined as the complete resolution of diarrhoea within 72 hours of randomisation into the study.

Clinical failure

Defined as the lack of resolution or worsening of diarrhoea, within 72 hours of randomisation into the study, or any patient requiring >2 unscheduled IV infusions.

Oral therapy success

Defined as the complete resolution of all signs or symptoms of dehydration, such that no i.v. rehydration had to be restarted.

Oral therapy failure

Defined as the reappearance of the signs and/or symptoms of dehydration, such that intravenous fluid treatment had to be restarted.

Clinical outcome indeterminate:
A valid assessment of clinical efficacy can not be made or a response cannot be obtained due to the patient being withdrawn from the study due to an adverse event poor patient co-operation or extenuating circumstances.

5.4.7 Study Conclusion

The study conclusion page in the CRF must be completed for all patients registered onto the study irrespective of whether they have completed the treatment period or were withdrawn from the study.
All patients that enter the study must have this page completed.

6.0 STUDY MEDICATION

6.1 Dosage and Formulation

Racecadotril / Hidrasec™ will be supplied as ivory coloured 100mg capsules

Placebo will be supplied as identical ivory coloured capsules

A single dose of Doxycycline 300mg will be administered to all patients randomised onto the study.

SmithKline Beecham will provide the blinded medication to the centre and the Doxycycline 300mg will be sourced locally.

6.2 Study drug administration

On admission to the hospital, patients eligible to enter the study will be rehydrated to replace the fluid deficit; this will be achieved by intra venous and oral fluids. The volume of i.v. fluid given should be calculated on 5% - 10% of the body weight. At the end of the four to six hour observation period, consenting patients with a purging rate of ≥ 5ml/kg/hr body weight and patients that no longer require IV fluid replacement will be randomised into one of two groups:

Group A - patients will receive racecadotril 100 mg (1 capsule) 6 times a day (every 4 hours)
Group B - patients will receive placebo (1 capsule) 6 times a day (every 4 hours)

Treatment will be continued until recovery, (12 hours with no stools or 2 consecutive normal stools), or for a maximum of 3 days.

Re-dosing:
If the patient vomits within 10 minutes of administering the study medication they must be re-dosed. If a patient vomits on 2 consecutive occasions they must be withdrawn from

the study and considered a treatment failure. If the patient vomits within 10 minutes of administering the stated dose of Doxycycline then they can be re-dosed.

6.3 Blinding

The blinding/randomisation of the medication is determined when the individual patient study supplies are packaged at SmithKline Beecham, France. The study centre is required to dispense the patient medication in chronological order to each of the patients entering the study. A sealed envelope containing the randomisation code breaks will be given to the Investigator for safe keeping to enable easy access in the event of an emergency, requiring the code to be broken.

6.3.1 Management of Emergency Code Break

The emergency code breaks will be placed by SB in a sealed envelope and kept at the study centre. The code must only be broken in the case of medical events which the investigator/physician in charge of the patient feels cannot be adequately treated without knowing the identity of the study medication.

Every effort should be made to contact the SB Medical Monitor prior to breaking the code. If it is not possible, and the situation is an emergency, the investigator may break the code and contact the SB Medical Monitor as soon as possible.

The following information needs to be recorded in the event of an Emergency Code Break:

- Date of Code Break
- Identification of person(s) requesting the Code Break
- Reason for breaking the code
- Investigator Signature.

The person who broke the code must sign and date the envelope which, if opened at study site, will be returned to SB by the SB site monitor. The patient will be withdrawn from the study.

6.4 Concomitant Medication/Treatment

Any concomitant medication taken during the study must be recorded in the case report form with the indication, the name of the preparation, the date(s) of administration, the route of administration and the total daily dose. If a patient in the clinician's opinion requires antibiotic or antidiarrhoeal treatment at any stage during the study, in addition to the stat dose of doxycycline given at randomisation, they must be withdrawn from the study immediately and considered a treatment failure.

6.4 Packaging

The Investigator will receive sufficient study medication for each of his patients. One package of study medication will be allocated for each patient. This will contain 4 separate blister packs, each of which will contain 6 tablets.
Each blister will be labelled Day 1, Day 2, Day 3 or Re-dosing Blister.

6.5 Labelling and preparation

PATIENT PACK LABEL: DAY 1 - 3

Study No: 52607/002 **Patient Number: XXX**
Contains : 24 capsules
Batch No: **Expiry Date:**
Manufacturing Date:

Administer capsules by mouth as directed.

For Clinical Trial Use Only
KEEP OUT OF REACH OF CHILDREN
PROTECT FROM LIGHT
STORE IN DRY CONDITIONS BETWEEN 15 ° C - 30 ° C
SmithKline Beecham Pharmaceuticals
SB House, Great West Road
Brentford, Middlesex, England.

TEAR-OFF PORTION OF LABEL

Study No: 52607/002 **Patient No: XXX**

Dispense on Day 1

Instructions: Tear off this portion of label and fix it securely to the designated area of the CRF.

INDIVIDUAL BLISTER PACK

Study No: 52607/002 **Patient No: XXX**

Batch No: **Expiry Date:**
Manufacturing Date: **DAY 'n'**

Administer capsules by mouth 1 capsule 6 times daily

For Clinical Trial Use Only
KEEP OUT OF REACH OF CHILDREN
STORE IN DRY CONDITIONS BETWEEN 15 ° C - 30 ° C
SmithKline Beecham Pharmaceuticals
SB House, Great West Road
Brentford, Middlesex, England.

Study No: 52607/002 **Patient No: XXX**

Batch No: **Expiry Date:**
Manufacturing Date: **Redosing Blister**

Administer as specified in the protocol

For Clinical Trial Use Only
KEEP OUT OF REACH OF CHILDREN
STORE IN DRY CONDITIONS BETWEEN 15 ° C - 30 ° C
SmithKline Beecham Pharmaceuticals
SB House, Great West Road
Brentford, Middlesex, England.

6.6 Storage

All medication must be stored in a locked cabinet under controlled room temperature.

6.7 Drug Accountability

The Investigator or pharmacist will sign to indicate that the clinical supplies for the study have been received. The investigational products must be handled and described in the sections 6.2 to 6.6 and dispensed only to those patients enrolled in the study.

At the end of the study it is necessary to reconcile delivery records with those of usage and returned stock. Account must be given of any discrepancies. A drug Disposal Form must be signed with the assurance from the Investigator /Pharmacist that all unused medication for the study will be returned, or destroyed locally, including all opened and unopened packages (according to the directions of the SB Monitor)

6.8 Assessment of Compliance

A record of the number of capsules dispensed, taken and returned for each patient must be documented in the CRF. Compliance will be assessed from information recorded in the CRF, including capsule count and start and end date of therapy.

6.9 Treatment of overdosage

The dosage of Racecadotril considered to be an overdose in humans has not been defined. Acute overdosage of Racecadotril in humans has not been reported and no specific treatment is known. If overdosage is suspected, patients should be treated as clinically indicated.

7.0 ADVERSE EXPERIENCES

The recording of adverse experiences is an important aspect of study documentation. Detailed guidelines are given below.

7.1 Eliciting, and documenting adverse experiences

It is responsibility of the investigator to document all adverse experiences, which occur during the investigation.

An adverse experience includes any noxious, pathological or unintended change in anatomical, physical or metabolic functions as indicated by physical signs, symptoms and/or laboratory changes occurring in any phase of the clinical study whether associated with the study medication, active comparator or placebo and whether or not considered drug related. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the disease under investigation that is not recorded elsewhere in the case report form under specific efficacy assessments. Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered adverse experiences. Discrete episodes of chronic conditions occurring during a study period should be reported as adverse experiences in order to assess changes in frequency or severity.

Adverse experiences should be documented in terms of a medical diagnosis(es). When this is not possible, the adverse experience should be documented in terms of signs and/or symptoms observed by the Investigator or reported by the patient at each study visit.

Any pre existing conditions or signs and/or symptoms present in a patient prior to the start of the study should be recorded on the Medical/Surgical history form within the patients' case report form (CRF).

Adverse experiences that occur after informed consent is obtained, but prior to starting active or randomised treatment will be documented on the 'Baseline signs and symptoms' page in the CRF. All adverse experiences occurring after administration of the first dose of study medication (i.e. active drug, placebo, comparator drug) must be reported on the

Adverse Experience page in the subjects CRF. All subsequent adverse experiences, whether no drug (i.e. during reference 'run-in' or 'wash-out' period) or when active drug or placebo is being administered, must be reported **REGARDLESS OF WHETHER OR NOT THEY ARE CONSIDERED DRUG RELATED.**

Serious adverse experiences which occur during the clinical study or within 30 days or five half lives, whichever is the longer of receiving the last dose of study medication, whether or not related to study drug, must be reported.

Instances of death, cancer or congenital abnormality if brought to the attention of the Investigator AT ANY TIME after cessation of study medication AND considered by the Investigator to be possibly related to study medication, should be reported to the site monitor.

At each visit/assessment, the investigator will evaluate adverse experiences. Adverse experiences not previously documented in the study will be recorded in the adverse experience section of the subject's CRF. The nature of each experience, date and time (where appropriate) of onset, outcome, course (i.e. intermittent or constant), duration, severity and relationship to treatment should be established. Details of changes to the dosage schedule or any corrective treatment should be recorded on the appropriate pages of the CRF.

Adverse experiences already documented in the CRF, (i.e.at a previous assessment) and designated as "continuing" should be reviewed at subsequent visits as necessary. If these have resolved, the documentation in the CRF should be completed. NB: If an adverse experience changes in frequency or severity during a study period, a new record of the experience will be started.

As a consistent way of soliciting adverse experiences, ask the subject a non-leading question such as:

"Have you felt or appeared different in any way since starting the new treatment/ or since the last visit?"

7.2 Assessment of Severity

Maximum severity should be assigned to one of the following categories:

Mild
An adverse experience, which is easily tolerated by the subject, causing minimal, discomfort not interfering with everyday activities.

Moderate
An adverse experience which is sufficiently discomforting to interfere with normal everyday activities.

Severe
An adverse experience which prevents normal everyday activities.

7.4 Assessment of causality

Every effort should be made by the investigator to explain each adverse experience and assess its relationship, if any, to study drug treatment. Causality should be assessed using the following categories: not related, unlikely, suspected (reasonable possibility) or probable.

Not related: The adverse experience is definitely not related to the test drug.

Unlikely: There are other, more likely causes and the drug is not suspected as the cause.

Suspected (reasonable possibility): A direct cause and effect relationship between the drug and the adverse experience has not been demonstrated but *there is a reasonable possibility that the experience was caused by the drug.*

Probable: There *probably* is a direct cause and effect relationship between the adverse experience and the study drug.

The degree of certainty with which an adverse experience is attributed to drug treatment (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the experience can be understood in terms of one or more of the following:

- Known pharmacology of the drug
- Reaction of similar nature being previously observed with this drug or class of drug
- The experience having often been reported in literature for similar drug as drug related, e.g., skin rashes, blood dyscrasia.
- The experience being related by time to drug ingestion terminating with drug withdrawal (dechallenge) or reproduced on rechallenge.

7.4 Following-up of adverse experiences

Investigators should follow-up subjects with adverse experiences until the event has subsided (disappeared) or until the condition has stabilised. Reports relative to the subject's subsequent course must be submitted to the clinical study monitor.

7.5 Serious Adverse Experiences

7.5.1 Definition of Serious Adverse Experiences:

A serious adverse experience is any event which is fatal, life threatening, disabling/incapacitating or results in hospitalisation, prolongs a hospital stay or is associated with congenital abnormality, cancer or overdose (either accidental or intentional). In addition, any experience which the investigator regards as serious or which would suggest any significant hazard, contraindication, side effect or

precaution that may be associated with the use of the drug should be reported as a serious event.

Life threatening definition:

An adverse experience is life threatening if the subject was at immediate risk of death from the event as it occurred (i.e., it does not include a reaction that if it had occurred in a more serious form might have caused death). For example, drug induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening even though drug- induced hepatitis can be fatal.

Disability/incapacitating - definition:

An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions.

Hospitalisation – definition

An adverse experience requiring hospitalisation should be considered serious. Hospitalisation for elective surgery or routine clinical procedures that are not a result of an AE (e.g. elective surgery for a pre-existing condition) need not be considered AE's and should be recorded on the medical/ surgical procedures form. If anything untoward is reported during the procedure, that occurance must be reported as an AE, either 'serious' or 'non-serious' according to the usual criteria.

In general, hospitalisation signifies that the subject has been detained (usually involving at least one overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. When in doubt as to whether 'hospitalisation' occurred or was necessary, the adverse experience should be considered serious.

7.5.2 Reporting Serious Adverse Experiences

Any serious adverse experiences which occur during the clinical study or within 30 days (or five half-lives, whichever is longer) of receiving the last dose of medication, whether or not related to the study drug, must be reported by the investigator to the study monitor by telephone within 24 hours.

All serious adverse experiences must be reported by telephone within 24 hours to the study monitor:

Name: Please complete appropriate information locally.

Address: Please complete appropriate information locally.

Phone number: Please complete appropriate information locally.

Alternate(s)

Investigators should not wait to receive additional information to fully document the event before notifying SmithKline Beecham of a serious adverse experience. The telephone report should be followed by a full written summary detailing relevant aspects of the adverse experience in question. Where applicable, information from relevant hospital case records and autopsy reports should be obtained.

Instances of death, cancer or congenital abnormality if brought to the attention of the investigator **at any time** after the cessation of study medication **and** considered by the investigator to be possibly related to study medication, should be reported to the study monitor.

7.6 Reporting of Overdose

Any instance of overdosage (suspected or confirmed and irrespective of whether or not it involves study medication) must be communicated to SmithKline Beecham within 24 hours and be fully documented as a serious adverse experience. Details of any signs or symptoms and their management (see section 6.9) should be recorded including details of any antidote(s) administered.

7. 7 Pregnancy

Patients should be instructed to notify the investigator if it is determined after completion of the study of the study that they became pregnant within 30 days after the treatment period.

Whenever possible a pregnancy should be followed to term, any premature termination reported, and the status of the mother and child should be reported to SB after delivery.

8.0 SUBJECT COMPLETION AND WITHDRAWAL

8.1 Definitions

A patient is defined as having completed the study if they have:

- Completed the treatment period either by concluding complete resolution of diarrhoea (defined as 12 hours with no stools or 2 consecutive normal stools)
- Completed a maximum of 3 days study medication followed by a 24 hour-observation period.

A patient is defined as having withdrawn from the study if they were:

- Randomised to receive study medication but did not complete the study (irrespective of whether they actually received study medication).

8. 2 Procedures for handling withdrawals

When a patient is withdrawn, the investigator should carry out all the assessments that would have been carried out at the next scheduled visit (unless the patient is lost to follow-up).

Patients withdrawing from the study should attend a follow-up visit three weeks after withdrawal for the monitoring of adverse experiences and changes in concomitant medication. Every effort will be made to follow-up patients who withdrew due to drug related adverse experiences in order to determine the final outcome. This must then be recorded in the CRF and reported to SB pharmaceuticals. The study conclusion page of the CRF must be completed for all patients taking part in this study.

8.3 Reasons for withdrawal

The investigator may stop medication and withdraw the patient or the patient may withdraw him/herself from the study at any time. The reason for withdrawal must be recorded in the CRF.

Reasons for withdrawal include:

- **Adverse Experience:**
  Patient has any adverse experience deemed sufficiently severe to warrant withdrawal. This must be recorded in the CRF and all adverse experiences followed-up until resolution.

- **Insufficient therapeutic effect:**
  In the opinion of the investigator there has been a clinical failure of study medication on-therapy and further treatment is required.

- **Protocol deviation**
  Non compliance with study medication or visit schedule.
- **Patient is lost to follow up**
- **Termination by sponsor**
- **Other**
  Patient withdraws consent or requests cessation of treatment

9.0 DATA EVALUATION

There will be two sets of analysis performed on data in this study. 'Statwood', Hitchin, Hertfordshire, England, will perform data management for SmithKline Beecham, United Kingdom, and ICDDR,B will perform an independant analysis within their hospital. Every effort will be made to ensure that both sets of data are identical, at both locations. In the event of any discrepancies occurring, both parties (Statwood and ICDDR,B) in the presence of SmithKline Beecham will review the data file and come to a reasonable solution which is scientifically valid.

9.1 Criteria for Efficacy

9.1.1 Primary Efficacy Variable

To assess the total stool output in g/kg bodyweight from the initiation of randomised treatment until either the resolution of diarrhoea, or the time of discharge, whichever is the sooner.

9.1.2 Secondary Efficacy Variables

- Total stool output in g/kg at 24 and 48 hours
- Duration of diarrhoea
- Intake of ORS and plain water, in ml/kg of body weight, from initiation of treatment until the end of diarrhoea or the end of study (3 days).
- Weight gain, expressed as percent increase in weight, from start of study medication until the resolution of diarrhoea or the end of study
- Occurrence of hypernatraemia or hyponatraemia.

At the end of the study, the investigator will evaluate each patient's clinical response to the study medication with reference to the signs and symptoms at baseline, as follows:

Clinical Success:
Defined as the complete resolution of all signs and symptoms of acute diarrhoea such that no additional therapy is required.

Clinical Failure:
Defined as the lack of resolution or worsening of the signs and/or symptoms of acute diarrhoea, such that study medication was stopped and alternative therapy was given

Oral Therapy – Success:
Defined as the complete resolution of all signs and/or symptoms of dehydration such that no i.v. rehydration had to be restarted.

Oral Therapy – Failure:
Defined as the reappearance of all the signs and/or symptoms of dehydration, such that intravenous fluid treatment had to be restarted.

Unable to determine:
A valid assessment of clinical efficacy can not be made (e.g., patient did not attend the end of therapy visit) or a response cannot be obtained due to poor patient co-operation or extenuating circumstances.

9.2 Statistical Methods

9.2.1 Comparisons of interest

Primary Outcome

The primary interest in this study is to assess the total stool output during the study. That is, the total output from the time of starting randomised medication until either the diarrhoea has resolved, or until the time of discharge, whichever is the sooner

Secondary Outcome

The secondary objectives in this study are the following:

- Stool output for the first 24 and 48 hours following start of study medication
- Duration of diarrhoea
- Total intake of ORS and plain water from the start of study medication until the resolution of diarrhoea, or the end of study
- Weight gain, expressed as percent increase in weight after resolution of diarrhoea, or at the end of study, compared with weight on randomisation
- The occurrence of hypernatraemia or hyponatraemia
- The overall response to therapy, as either clinical success or clinical failure, and oral rehydration success or oral rehydration failure.

9.2.2 Sample Size Determination

A total of 110 adult patients are to be enrolled to accrue 100 evaluable ITT patients with acute diarrhoea. The study has been designed to demonstrate a reduction of 33% in the total stool output for Racecadotril compared to placebo. The sample size calculation assumed a control mean of 273 g/kg with a standard deviation of 157 g/kg (see Alam et al, Lancet 1999). The study has been designed to have 80% power to detect this difference using a two-tailed, 5% level of significance. It is estimated that 50 patients would be

required in each group, but in order to allow for a dropout rate of approximately 10%, 110 patients will be enrolled.

9.2.3 Interim Analysis

There will be no interim analysis.

9.3 Efficacy Analysis

9.3.1 Methods of Analysis

The baseline demographic characteristics will be summarised using descriptive statistics to facilitate evaluation of possible differences between treatment groups, which might bias the comparison of treatments. Study medication compliance, protocol violations and reasons for dropouts will be examined both as indicators of treatment differences and to evaluate any biases in the proposed treatment comparisons.

The primary outcome variable (see section 9.2.1) will be compared between groups using analysis of variance. The difference between Racecadotril and placebo will be estimated using the mean difference with its accompanying 95% confidence interval. The effects of age and sex will be included in the model as covariates. If the effects do not achieve significance at the 5% level they will be dropped. Plots of residuals will be examined to check the validity of the assumptions of normal distribution and stable variance.

In the event that either or both of these assumptions are not met, a data transformation such as the logarithmic transform, will be considered. The model may also be fitted using total stool output as the outcome variable, with bodyweight at start of treatment as a covariate to see whether the model fit is improved. In the event that a logarithmic transformation is used, the results will be expressed as the ratio of the geometric means with accompanying 95% confidence interval. If no suitable transformation can be found, non-parametric methods such as the Wilcoxon test will be considered.

The analysis of the secondary outcome variables i.e. stool output for the first 24 hours and the first 48 hours will be analysed in a similar way, as will the data on ORS and water intake. The duration of diarrhoea will be compared between treatments using lifetable methods, with the logrank test, or Gehan test used as appropriate. The percentage increase in weight from start of study medication until the end of study will be compared between treatments using analysis of covariance, with the baseline weight as the covariate. If plots of residuals indicate that distribution assumptions may be in doubt, then the absolute weight change will be analysed instead. Data transformation will be considered, as above, if residual plots indicate a problem.

The incidence of clinical success will be compared between treatments using a chi-squared test, as will the incidence of oral therapy success. The difference between treatments will be expressed as the difference between proportions of successful outcomes with associated confidence interval.

In order to take account of the fact that there are several secondary outcomes, to avoid the problem of multiplicity, the significance level to be used for all secondary outcome analyses will be 1%, with associated 99% confidence intervals for differences between

treatments.

Data will be also be summarised in graphs, where appropriate, and tables

9.3.2 Patient Population/Data Sets to be Evaluated

i) Intent to-treat population

All patients with a valid baseline and who have taken at least one dose of the study medication will qualify for the intent-to-treat analysis. All such patients with at least one on-treatment assessment of stool output will contribute data to the full analysis set. This will form the primary analysis set.

ii) Per-protocol population

The per-protocol analysis of efficacy will be used to confirm the findings from the intent-to-treat population. Patients will be eligible for the per-protocol efficacy analysis providing the criteria in section i) above have been satisfied and the following apply:

- The patient has completed all assessments as specified by the protocol
- No major protocol violation exists with regard to Inclusion/Exclusion criteria
- A minimum of 80% compliance with study medication has been achieved
- No prohibited concomitant medications were taken during the treatment period

iii) Safety population

All patients who have received any study medication will be included in the safety population

9.4 Safety Evaluation

All adverse experiences reported following enrolment of a patient into the study will be documented. Signs and symptoms (other than those of the disease under investigation) which occur prior to the first dose of study medication will also be recorded.

Evaluation of safety data will be based on descriptive comparisons of adverse experiences between the treatment groups, with particular attention to the occurrence of constipation, defined as no stools during 36 or more consecutive hours since the last stool. Concomitant medication will also be summarised in tables

For adverse experience data the incidence of each experience will be described, but no formal analysis will be performed. Tables classifying events by body system, by severity, and by relationship to study drug will be produced where appropriate. Information

regarding concomitant medication will be presented as listings and tables.

10.0 ADMINISTRATIVE MATTERS

To comply with Good Clinical Practice important administrative obligations relating to investigators responsibilities, monitoring, archiving data, audits, confidentiality and publications must be fulfilled as given in Appendix B.

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WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians in
biomedical research involving human subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964,
Amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975,
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly Hong Kong, September 1989
and the
48th General Assembly, Somerset West, Republic of South Africa,
October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration", and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognised between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded with careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimise the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project, the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation. Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE
(Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

(Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers -- either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

APPENDIX B: ADMINISTRATIVE MATTERS

ADMINISTRATIVE MATTERS

I RESPONSIBILITIES OF THE INVESTIGATOR

- To ensure that he/she has sufficient time to conduct and complete the study and has adequate staff and appropriate facilities which are available for the duration of the study and to ensure that other studies do not divert essential subjects or facilities away from the study at hand.
- To submit an up-to-date curriculum vitae and other credentials (e.g. medical license number in the United States) to the sponsor and - where required - to relevant authorities.
- To acquire the normal ranges for laboratory tests performed locally and, if required by local regulations, obtain the Laboratory Licence or Certification.
- To prepare and maintain adequate case histories designed to record observations and other data pertinent to the study.

II PROTOCOL AMENDMENTS

No changes to the study protocol will be allowed unless discussed in detail with the SmithKline Beecham (SB) Medical Monitor and filed as an amendment/modification to this protocol.

Any amendment/modification to the protocol will be adhered to by the participating centre (or all participating centres) and will apply to all subjects following approval as appropriate by the Ethical Review Committee or Institutional Review Board.

III SPONSOR'S TERMINATION OF STUDY

SB reserves the right to discontinue the clinical study at any time for medical or administrative reasons. When feasible, a 30-day written notification will be tendered.

IV CASE REPORT FORM INSTRUCTIONS

Prior to screening the first potential participant, the investigator will provide a list showing the signature and hand-written initials of all individuals authorised to make or change entries on case report forms (CRFs). If the authorised individuals should change during the study, the investigator is to inform SB.

CRFs (and subject diary cards, if applicable), will be supplied by SB for recording all data. It is the responsibility of the investigator or co-investigator to ensure that CRFs (and subject diary

cards) are legible and completely filled in with a black ink ball-point pen. Include the subject's identification (2-3 alphabetic letters representing the subject's initials or the first 2-3 letters of subject's first or last name), the patient identification (PID) if not already pre-printed and the visit date, will be entered on the CRF Header Pages as required.

Errors must be corrected by drawing a single line through the incorrect entry and writing in the new value/data positioned as close to the original as possible. The correction must then be initialled, dated and justified by the authorised individual making the change. Do not obliterate, write over, or erase the original entry when making a correction.

When a subject completes a visit, it is anticipated that relevant sections of the CRF will be completed by the investigator (or designated staff) within 24 hours of the last data becoming available, but in no case later than 5 days. Similarly, when a subject completes a study, it is anticipated that all relevant CRF pages will be completed within 24 hours of the last data becoming available, but in no case later than 5 days. This also applies to forms for potential study participants who were not randomised to a treatment group.

As soon as the subject has completed/withdrawn from the study and the CRF is completed the principal investigator or designated physician(s) under his/her supervision will sign the adverse experience page(s) as well as study conclusion page of the CRF to confirm that they have reviewed the data and that the data are completed and accurate. If sections of a CRF are to be brought into SB prior to study conclusion, a section conclusion signature is required.

An original (top copy) of the CRF must be submitted for all subjects who have undergone protocol specific procedures, whether or not the subject completed the study.

While completed CRFs will be reviewed by an SB professional monitor at study site, errors detected by subsequent in-house CRF review may necessitate clarification or correction of errors and documentation and approval by the investigator.

Any questions or comments related to the CRF should be directed to the assigned study monitor.

V MONITORING BY SMITHKLINE BEECHAM

Monitoring visits by a professional representative of the sponsor will be scheduled to take place before entry of the first subject, during the study at appropriate intervals and after the last subject is completed.

These visits are for the purpose of verifying adherence to the protocol and the completeness and exactness of data entered on the CRF and Drug Inventory Forms. The monitor will verify CRF entries by comparing them with the hospital/clinic/office records which will be made available for this purpose. The monitor will retrieve completed CRF sections at each visit. Adequate time and space for these visits must be made available by the investigator.

Investigator must ensure provision of reasonable space and adequate qualified personnel for monitoring visits.

VI ARCHIVING OF DATA

The investigator must retain subject records and CRFs as well as drug disposition records in an easily retrievable form until disposal has been agreed in writing with SB. The investigator must have a 'key' linking the subject's study identification number (i.e. treatment number) to the subject's clinical file. If the investigator moves or retires, he/she must nominate someone in writing to be responsible for record keeping. Archived data may be held on microfiche or electronic record, provided that a back-up exists and a hard copy can be obtained from it if required.

SB agrees to retain a copy of the protocol, documentation, approvals and all other documents related to the study, including certificates that satisfactory audit and inspection procedures have been carried out.

VII AUDITS

For the purpose of compliance with Good Clinical Practice and Regulatory Agency Guidelines it may be necessary for SB or a Drug Regulatory Agency to conduct a site audit. This may occur at any time from start to after conclusion of the study.

When an investigator signs the protocol, he agrees to allow the Drug Regulatory Agency and SB auditors to inspect his/her study records. Furthermore, if an investigator refuses an inspection, his data will not be accepted in support of a New Drug Registration and/or Application.

SB has a substantial investment in clinical studies. Having the highest quality data and studies are essential aspects of drug development. SB has a Regulatory Compliance staff who audit investigational sites. Regulatory Compliance assesses the quality of data with regard to accuracy, adequacy and consistency. In addition, Regulatory Compliance assures that SB sponsored studies are in accordance with the relevant Good Clinical Practices regulations/guidelines are being followed.

To accomplish these functions, Regulatory Compliance selects investigational sites to audit. These audits usually take 1 to 2 days. The SB audits entail review of source documents supporting the adequacy and accuracy of CRFs, review of documentation required to be maintained, and checks on drug accountability. The SB audit therefore helps prepare an investigator for a possible regulatory agency inspection as well as assuring SB of the validity of the database across investigational sites.

The Inspector will be especially interested in the following items:

- Log of visits from the sponsor's representatives
- ERC/IRB approval
- Test article accountability
- Approved study protocol and amendments
- Informed consent of the subjects (written or witnessed oral consent)
- Medical records supportive of CRF data
- Reports to the ERC/IRB and the sponsor
- Record retention

SB will gladly help investigators prepare for an inspection.

VIII CONFIDENTIALITY AND PUBLICATION

You agree that all information communicated to you by SB is the exclusive property of SB and you will ensure that the same shall be kept strictly confidential by you or any other person connected with the work and shall not be disclosed by you or such person to any third party without the prior written consent of SB. You shall communicate the results of the work promptly to SB.

We agree that you shall have the right to publish or permit the publication of any information or material relating to or arising out of the work after prior submission to us provided that if we shall so request you will delay publication for a maximum of six months to enable us to protect our rights in such information or material. Any proposed publication or presentation (e.g. manuscript, abstract or poster) for submission to a journal or scientific meeting, should be sent to the study monitor prior to submission. SB will undertake to comment on such documents within four weeks.

All rights and interests world-wide in any inventions, know-how or other intellectual or industrial property rights which arise during the course of and/or as a result of the clinical study which is the subject of the Protocol or which otherwise arise from the information or materials supplied under this Agreement, shall be assigned to, vest in and remain the property of SmithKline Beecham plc.

APPENDIX C Voluntary Consent Form

International Centre for Diarrhoeal Disease Research, Bangladesh

Title of the Research Project: A double-blind randomized placebo controlled, parallel group study to assess the efficacy, safety, and tolerability of RACECADOTRIL (HIDRASEC™) in the treatment of acute diarrhoea resulting from *V. cholerae* in adults

Principal Investigator: DR. Nur Haque Alam

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

You are suffering from acute watery diarrhoea caused by *Vibrio cholerae* (Cholera). The mainstay of treatment of this disease is the assessment of dehydration and the appropriate replacement of fluid and electrolytes. In addition antibiotic and normal food are given. ICDDR,B is carrying out a study to determine whether Racecadotril (Hidrasec™) would be beneficial in terms of reducing stool output and duration of diarrhoea. The study will also provide further data on the safety and tolerability of Hidrasec™. It is hoped that this drug will help reducing disease severity and early recovery. The safety profile of Hidrasec™ has demonstrated that it is well tolerated and is known to be safe at this time.

We would be grateful if you would agree to participate in this study, you would be involved on the study for a maximum of 4 days. If you do, you can expect that the following procedures will be done

1.You will be asked some questions about your medical history and a thorough physical examination will be performed.

2.Blood (5ml) will be drawn from your vein for some tests. Your stool sample will be taken for culture on admission and daily for 4 days.

3.After intravenous hydration, you will be given ORS to drink, Cap doxycycline 300 mg once and usual hospital diet. You may get study drug (Hidrasec™) or placebo, according to the randomization number.

4.After discharge from the hospital you may be asked to come for follow up visit. During this visit you will be asked about any symptoms you have and a physical examination will be done.

5.Your participation in the study is entirely voluntary. Your refusal to agree to participation in the study will involve no penalty or loss of benefits to which you are otherwise entitled. During the study, you may discontinue your participation. Even then your treatment in the hospital will be continued as usual, and your relationship with the doctor and other staffs will also be normal as before.

If you have any questions concerning the study, please contact:

Name:	Telephone Number
1. Dr. Nur Haque Alam	2311
2. Dr. Wasif Ali Khan	2333
3. Dr. Hasan Ashraf	2313

7.Your participation in this study will be treated in the strictest confidence. Medical records will need to be reviewed by SB personnel eg. authorised representatives of the drug company or regulatory authorities may need to inspect your notes, however, as far as possible, your confidentiality will be maintained. The Company will ensure that it complies with any national data protection regulations.

8.If you become ill as a result of participation in this clinical study, medical treatment will be provided and the reasonable costs of such treatment will be paid by the Company.

If you agree to participate in the study, please sign or give your thumb impression on the form. A copy of this form will be given to you for your information.

....
Signature/thumb impression of the patient Date

....
Signature of the Investigator Date

....
Signature of the witness Date

.....
Patients Study Number

APPENDIX D: ASSESSMENT OF DEHYDRATION

	A	B	C
Condition	Well, alert	***Restless, irritable**	***Lethargic or unconscious; floppy**
Eyes	Normal	Sunken	Very sunken and dry
Tears	Present	Absent	Absent
Mouth and Tongue	Moist	Dry	Very dry
Thirst	Drinks normally, not thirsty	***Thirsty, drinks eagerly**	***Drinks poorly or not able to drink**
Skin pinch	Goes back quickly	***Goes back slowly**	***Goes back very slowly**
Decide:	The patient has NO SIGNS OF DEHYDRATION	If the patient has two or more signs including at least one ***sign***, there is MODERATE DEHYDRATION	If the patient has two or more signs, including at least two ***signs***, there is SEVERE DEHYDRATION

The signs in **bold** with (*) are the most valuable for assessing dehydration; these are called 'key signs'

APPENDIX E : SCHEDULE OF ASSESSMENT

PROCEDURE	HOUR -6 / -4	Day 1 HOUR 0	Day 1 HOUR 4	Day 1 HOUR 8	Day 1 HOUR 12	Day 1 HOUR 16	Day 1 HOUR 20	Day 1 HOUR 24	Day 2 HOUR 28	Day 2 HOUR 32	Day 2 HOUR 36	Day 2 HOUR 40	Day 2 HOUR 44	Day 2 HOUR 48
Informed Consent	X													
Diagnosis	X	X												
Medical History	X													
Physical Examination	X													
Prior/Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Baseline signs and symptoms	X													
Assessment of vomiting	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of rehydration		X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of weight/vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of stool output	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of dehydration	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of IV fluid infused		X	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~
Assessment of water intake		X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of ORS intake		X	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of urine output		X	X	X	X	X	X	X	X	X	X	X	X	X
Collection of stool samples	X							X♦	X♦	X♦	X♦	X♦	X♦	X♦
Collection of blood samples		X						X*						X♦
Dispense study medication		X												
Adverse Experiences		X	X	X	X	X	X	X	X	X	X	X	X	X

* Only for subjects who had clinically abnormal findings during the study. Bloods must be taken at screening and discharge

~ Only for subjects that are recommenced on IV therapy after randomisation

♦ Stool samples to be collected once every 24 hours and then 24 hours after resolution of diarrhoea if possible.

APPENDIX E : SCHEDULE OF ASSESSMENT

PROCEDURE	Day 3 HOUR 52	Day 3 HOUR 56	Day 3 HOUR 60	Day 3 HOUR 64	Day 3 HOUR 68	Day 3 HOUR 72	Day 4 HOUR 76	Day 4 HOUR 80	Day 4 HOUR 84	Day 4 HOUR 88	Day 4 HOUR 92	Day 4 HOUR 96
Informed Consent												
Diagnosis												
Medical History												
Physical Examination												
Prior/Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X
Baseline signs and symptoms												
Assessment of vomiting	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of rehydration	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of weight/vital signs	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of stool output	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of dehydration	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of IV fluid infused	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~	X~
Assessment of water intake	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of ORS intake	X	X	X	X	X	X	X	X	X	X	X	X
Assessment of urine output	X	X	X	X	X	X	X	X	X	X	X	X
Collection of stool samples	X♦	X♦	X♦	X♦	X♦	X♦	X♦	X♦	X♦	X♦	X♦	X♦
Collection of blood samples						X*						X*
Dispense study medication												
Adverse Experiences	X	X	X	X	X	X	X	X	X	X	X	X

* Only for subjects who had clinically abnormal findings during the study. Bloods must be taken at screening and discharge

~ Only for subjects that are recommenced on IV therapy after randomisation

♦ Stool samples to be collected once every 24 hours and then 24 hours after resolution of diarrhoea if possible.

Detailed Budget for New Proposal

Project Title : A double-blind randomized controlled .....HIDRASEC in the treatment of cholera in adults

Protocol Number: **Name of Division: Clinical Sciences Division**

Funding Source: **Smith Kline Beecham**
Amount Funded (Direct) **$ 85,286** **Overhead (25%): $ 21,322** **Total: 106,608**

Staring Date: As soon as fund is available **Closing Date: 15 months from starting**

Strategic Plan Prioprity Code (s): **11**

Sl #	Account Description	Salary Support			US $ Amount Requested		
	Personnel	Position	Effort %	Time frame (months)	2000 1st yr.	2000 2nd yr.	Total
1	Dr. NH Alam (PI)	Sr. Medical Officer	30	15	3,350	3,045	6,395
2	Dr. GJ Fuchs (Co-PI)	Div. Director	10	15	9,080	8,260	17,340
3	Dr. WA Khan (Co-Inv)	Assist. Scientist	10	15	765	700	1,465
4	Dr. H Ashraf (Co-Inv)	Sr. Medical Officer	10	12	874	456	1,330
5	Study Medical Officer-1		100	10	3,146	818	3,964
6	Research Assistant-1		70	10	1,294	336	1,630
7	Study Nurses-4		100	10	2,833	736	3,569
8	Data Management Officer		30	10	912	240	1,152
9	Biostatistician-1		20	6	4,232	8,464	12,696
10	Secretary-1		20	15	999	910	1,909
	Sub Total				**27,485**	**23,965**	**51,450**
	Supplies and Materials (Description of items)						
1	Office stationary				200	300	500
2	Drugs and ORS				500	300	800
3	Non-stock supplies				200	100	300
	Sub Total				**900**	**700**	**1,600**
	Other Contractual Services						
1	Repair and Maintenance				100	100	200
2	Rent, Communications, Utilities (Email, fax, postal)				500	200	700
3	Printing and Publication				300	200	500
	Sub Total				**900**	**500**	**1,400**
	Interdepartmental Services						
1	Pathological tests						1,273
2	Microbiological tests						4,391
3	Biochemistry tests						2,772
4	X-rays, Blood cultures						100
5	Patient Study Ward (110 x 4 days x @45/day)						19,800
6	Local Transport						300
7	Xerox, Mimeographs etc						200
	Sub Total						**28,836**
	Other Operating Costs						
	Capital expenditure (computer and accessories, file cabinets)						2,000
	Sub Total						**2,000**
	Total Direct Cost						85,286
	Indirect cost (25%)						21,322
	TOTAL BUDGET COST						**106,608**

Detailed Budget of Laboratory Investigations				
Investigations	**Individual Cost**	**No.time required**	**No.of Patients**	**Total**
Pathological Tests				
Hematocrit, WBC (TC, DC)	2.30	3	110	759
Sp. Gravity	0.77	3	110	254
Stool microscopic exam	1.22	1	110	134
Dark-field exam	0.84	1	150	126
Sub total				**1,273**
Biochemistry Tests				
Electrolytes	4.40	3	110	1,452
Creatinine	2.20	3	110	726
BUN	1.80	3	110	594
Sub total				**2,772**
Microbiology Tests				
V.cholerae, Shigella, Salmonella, C. Jejuni (Including sensitivity charges)	18.47	1	110	2,032
V. cholerae	7.15	3	110	2,360
Sub total				**4,391**
Grand total				**8,437**

REVIEWER-A

Title: A double Blind randomized controlled clinical trial to study the efficacy, safety and tolerability of RACECADOTRIL (HIDRASEC) in the treatment of cholera in adults.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	✓		
Adequacy of project design		✓	
Suitability of methodology		✓	
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☒ - but see my questions + suggestions

b) with qualification

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: _____

Signature: ..... Date: Feb-2-00

Position: .

REVIEWER - A

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title: Clinical trial of Racecadotril in treatment of cholera

PI: Dr. Nur Haque Alam

Reviewer:

This study will test whether racecadotril, shown to be effective in other acute diarrheal syndromes, is effective in reducing the stool output + duration of diarrhea in cholera patients. The drug is certainly in need of testing + the cholera wards of ICDDR,B is an ideal place to do the study. The investigators are experienced in doing these kinds of studies.

I do have a few comments on the protocol. 1) The main one is the justification for the dose used in the study. Only a single dose will be used — but how this dose is chosen is not given. I presume it is based on drug effectiveness in previous studies. Certainly if any such drug is to be used in cholera treatment, it is important that the lowest effective dose be used - for reasons of price + acceptability. 2) Other comments - what types of side effects might be expected to be observed in this study? 3) There are a few ~~mis~~ mis-statements in the background a) Cholera is not endemic in the US b) the description of cholera including death within 2-3 hours + hypotension within 1 hr - these do not give an accurate picture of the disease. Usually 12 hrs to hypotension + possible death. c) The incubation period is usually 18-48 hrs. d) Electrolyte imbalances are not defined, + these are not usually complications.
4. Error in inclusion criteria - should be ≥ 20 ml/kg - not less than.
5. Follow up evaluation, page 8: When will this be?
6. Patient compliance, page 9. Should be "physician/nurse compliance" since presumably all the medication doses will be supervised.
7. Page 11 - I can't imagine "telephone reports" will be used as part of this study. (This protocol must have been copied in part ~~by~~ from the sponsor's protocol.)
8. Sample size calculations should be shown in more detail.
9. Data analysis - I think the term "qualitative data" is not used correctly.

Budget is appropriate.

Confidential

TO: Dr. George Fuchs
DIVISION DIRECTOR CSD
ICDDR, B, DHAKA
FAX: 00-8802-8823116 REVIEWER-B

Title: A double blind randomized controlled clinical trial to study the efficacy, safety and tolerability of RACECADOTRIL (HIDRASEC) in the treatment of cholera in adults.

Summary of Referre's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	✓		
Adequacy of project design	✓		
Suitability of methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge		✓	

CONCLUSIONS

I support the application:

a) without qualification ☑

b) with qualification

- on technical grounds ☐
- on level of financial support ☐

I do not support the application ☐

Name of Referee: .....

Signature: .....

Date: 21.2.2000

Position: .....

REVIEWER - B

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified.

(Use additional pages if necessary)

Title:

PI:

Reviewer:

Title: "A double-blind randomized _____of RACECADOTRIL (HIDRASEC)_____cholera in adults".
PI: Dr. NH Alam
Reviewer: D. Mahalanabis

Comments: The topic of research is of high scientific merit and deserves support. The project proposal is well written and the PI has extensive experience in conducting such studies. I fully support the research proposal. I have a few comments:

1. A summary of adverse effects from previous studies if any, should be provided.
2. Was any study done on the effect of Hydrasec on the intestinal transit time (in other words any study on the effect of the drug on the intestinal motility)?
3. Minor comment: on page 6 under Inclusion Criteria it should be 'more than/equal to' rather than 'less than/equal to'.
4. A copy of the master randomization code should be provided to the ICDDR,B for safe keeping and monitoring adverse effect. The code should of course be kept with some one not connected with the study.

I strongly support the study.

INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B. GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

To: Chairman
Research Review Committee

Date: March 6th 2000

Through: Director,
Clinical Sciences Division

From: Dr. Nur Haque Alam
Principal Investigator

Research protocol entitled: " A double blind, randomised, placebo controlled, parallel group study to assess the efficacy, safety and tolerability of Racecadotril in the treatment of acute diarrhoea resulting from *Vibrio cholerae* in adults".

Subject: **Response to External Reviewers' Queries/concerns**

We are pleased to enclose herewith our response to specific queries/concerns of the external reviewers of the proposal, for kind consideration of the Research Review Committee.

Response to Reviewer A

1. Justification for the dose (100 mg 6 times daily for 3 days)

Hidrasec with different dosage (30, 100 and 300 mg tds) has been evaluated in double blind clinical studies comparing placebo in acute diarrhoea. The study showed rapid efficacy of Hidrasec (all three dosages) in comparison with a placebo. However, there was no significance difference between the three different Hidrasec regiments. Although the diarrhoea was less severe in 30 mg receiving group.

This drug has not been evaluated in cholera patients, a disease consider being the most severe among the acute diarrhoea. Thus to determine its effect in cholera we have chosen a dose of 100 mg 6h for 3 days. If this dose is found to be effect in cholera patients a dose response study using lower dose regimens can be considered in future.

2. Side effect to observe

There are reports from clinical studies of minor side effects in association with Hidrasec. However, all these side effects encountered were comparable to the placebo group. These side effects includes:

GI tract- Nausea, vomiting, epigastric pain, constipation, abdominal bloating,
CNS - Dizziness, asthenia, headache, mood change, insomnia.
Skin - Skin rash, pruritus, acne
Uro-nephrology - Increase or decrease of urinary frequencies
Thus, we will look into those side effects and note in the CRF during the daily follow up. These side effects have been incorporated in the main protocol (p-10).

3. Minor corrections

a) Cholera is not endemic in USA. This line has been omitted (p-5, under subtitle rationale - 1st paragraph, line 7).

b) A healthy person may become hypotensive within few hours of diarrhoeal onset and death can occur from cholera as quick as within 12 hours of diarrhoeal onset (p-5, under subtitle rationale - 2nd paragraph, line 6).

c) Incubation period of *V. cholerae* is usually 18 to 48 hours (p-8, 3rd paragraph, line 1).

d) Acidosis is commonly observed in severely dehydrated cholera patients (p-8, 3rd paragraph, line 7).

4. Inclusion criteria

Purging rate ≥ 5 ml/kg/hr body weight during the four to six hour basal period (p-17; section 5.3.2).

5. Follow-up evaluation

Patients who were withdrawn from the study prior to the end of therapy will require a follow up safety assessment (not an efficacy assessment) at 28 days after the last dose of study medication (p-19; section 5.3.7).

6. Patient compliance

This sentence has been rephrased (p-26; section 6.8).

7. Telephone reports

During the patient recruitment period a regular monitor from the sponsor (*SmithKline Beecham*) will visit the recruitment site (Dhaka hospital; CRSC, CSD) and ensure that the GCP (Good Clinical Practice) guideline is followed. Thus if any serious adverse experience occurs during the clinical study or within 30 days of receiving the last dose of medication the investigator will have to report the study monitor by telephone within 24 hours (p-29, section 7.5.2). This is a guideline of GCP.

8. Sample size calculation

A more detailed description of sample size calculation is done (p-33, section 9.2.2).

9. Data analysis

The term qualitative data is omitted. A detailed description of the methods for data analysis is done. (P-34, section 9.3.1).

RESPONSE TO REVIEWER B

1. Adverse effects

This has been responded earlier (query no. 2 by reviewer A). The adverse effect has been incorporated in the main protocol (p-10). For more information on this product an Investigator brochure is also enclosed.

2. Intestinal transit time

There are studies shown that Hidrasec, by inhibiting enkephalinase it promotes the physiological activity of endogenous enkephalins and shows intestinal antisecretary activity without affecting intestinal transit time (ref. No. 16, 17, 18; p-38). As a result Hidrasec, has got added advantage over other antidiarrhoeal drugs like loperamide, which causes decreased intestinal motility and transit.

3. Inclusion criteria

This has been responded earlier (query no. 4 by reviewer A).

4. Master randomization code

We shall store the master randomization code of this study at ICDDR, B to some one who is not involved in the study. In fact this has been followed for quite sometime in all double blind randomized clinical studies conducted under CSD.

Thank you.

[R,S] ACETORPHAN
[R] ACETORPHAN

INVESTIGATOR BROCHURE

BIOPROJET
30, rue des Francs-Bourgeois
75003 PARIS
FRANCE

Offices :
9, rue Rameau
75002 PARIS
FRANCE

October 1997

1. INTRODUCTION

2. PHYSICOCHEMICAL CHARACTERISTICS

2.1 CHEMICAL NAME
2.2 DEVELOPED FORMULA
2.3 MOLECULAR FORMULA
2.4 CODE AND DENOMINATION
2.5 MOLECULAR WEIGHT
2.6 SOLUBILITY
2.7 DRUG PRODUCT
2.8 UNIT FORMULA
2.9 STABILITY
2.10 STORAGE CONDITION
2.11 SHELF-LIFE

3. TOXICOLOGICAL STUDIES

3.1 SINGLE DOSE TOXICITY
3.2 SUBACUTE TOXICITY
3.3 CHRONIC TOXICITY
3.4 REPRODUCTION STUDIES
3.5 SPECIFIC TOXICOLOGICAL STUDIES

4. ANIMAL PHARMACOLOGY

4.1 INTESTINAL ANTISECRETORY ACTIVITY
4.2 EFFECT OF [R,S]ACETORPHAN AND LOPERAMIDE ON GASTROINTESTINAL TRACT
4.3 OTHER ANTISECRETORY ACTIVITIES

5. PHARMACOKINETICS

5.1 ANIMAL PHARMACOKINETIC STUDIES

- ^{14}C-[R,S]ACETORPHAN : absorption, distribution, metabolism and excretion following a single oral dose to pigmented or non pigmented rats

- ^{14}C-[R,S]ACETORPHAN : absorption, distribution, metabolism and excretion following oral and intraveinous administration to the Cynomolgus monkey

5.2 HUMAN PHARMACOKINETIC STUDIES

- Study of the enkephalinase activity following a single oral dose of 30, 100 or 300 mg of [R,S]ACETORPHAN in man
- Pharmacokinetic study of [R]ACETORPHAN following a single oral dose of 50 mg in man
- Pilot pharmacokinetic study of [R,S]ACETORPHAN 100 mg versus [R]Acetorphan 75 mg tablet [5]
- Evolution of plasma enkephalinase activity after repeated administration of [R,S]ACETORPHAN in man
- Effect of food and Maalox on the pharmacokinetics of [R,S]ACETORPHAN after a single oral administration of 100 mg in healthy volunteers
- Pharmacokinetics of [R,S]ACETORPHAN in elderly
- Effect of [R,S]ACETORPHAN on Cytochrom P 450 activity
- Pharmacokinetics of [R,S]ACETORPHAN in association with loperamide or nifuroxazide

5.3 PHARMACOKINETICS IN CHILDREN

6. PHARMACODYNAMIC STUDIES

6.1 EFFECT OF [R,S]ACETORPHAN ON CASTOR-OIL INDUCED DIARRHEA IN MAN (Double-blind versus placebo study)

6.2 EFFECT OF [R,S]ACETORPHAN ON CHOLERA-TOXIN INDUCED DIARRHEA IN MAN

6.3 EFFECT OF [R,S]ACETORPHAN ON OROCAECAL AND COLONIC TRANSIT TIMES

6.4 EFFECT OF [R,S]ACETORPHAN ON GASTRIC ACID SECRETION

6.5 EFFECT OF [R,S]ACETORPHAN ON GASTRIC EMPTYING

- Effect of [R,S]ACETORPHAN on gastric emptying of a liquid meal
- Effect of [R,S]ACETORPHAN on gastric emptying of a solid meal

6.6 EFFECT OF [R,S]ACETORPHAN ON RECTAL AND ANAL MOTILITY

- Rectal electromyography
- Anorectal motility

6.7 EFFECT OF [R,S]ACETORPHAN ON ESOPHAGEAL MOTILITY IN HEALTHY VOLUNTEERS

6.8 COMPLEMENTARY PHARMACODYNAMIC STUDIES

- Effect of [R,S]ACETORPHAN on hormonal secretions in healthy volunteers
- Effect of [R,S]ACETORPHAN on hypothalamo-pituito-achenal axis in healthy volunteers
- Effect of [R,S]ACETORPHAN on bronchoconstriction in asthmatic patients
- Effect of [R,S]ACETORPHAN on vigilance

7. CLINICAL STUDIES

7.1 STUDIES IN ACUTE DIARRHEA

OPEN MULTICENTRIC STUDY

DOUBLE-BLIND VERSUS PLACEBO STUDIES

- Dose ranging study of [R,S]ACETORPHAN (30, 100 and 300 mg t.i.d.) in acute diarrhea
- French double-blind versus placebo study in acute diarrhea
- Tunisian double blind placebo controlled study

DOUBLE BLIND VERSUS LOPERAMIDE STUDIES

7.2 STUDIES IN CHRONIC DIARRHEA

- HIV+ diarrhea
- Chemotherapy-induced diarrhea

8. TOLERANCE

7.3 STUDIES IN PEDIATRICS

8.1 CLINICAL TOLERANCE

[R,S]ACETORPHAN

Phase I studies

Phase II and IV studies

Post marketing study

[R]ACETORPHAN

[R,S]ACETORPHAN : PEDIATRICS FORMULATION

8.2 BIOLOGICAL TOLERANCE

1. INTRODUCTION

[R] and [R,S]acetorphan are the first enkephalinase inhibitors used in therapeutics. [R,S]acetorphan obtained an approval from Health authorities for its therapeutic use in acute diarrhea.

The enkephalins, i.e. the first endogenous opiate substances discovered in 1975, play an important physiological role acting as neurotransmitters notably along the digestive tract. After their release and the stimulation of opiate receptors, the enkephalins are rapidly inactivated by enkephalinase. It was assumed that preventing this degradation would provide a therapeutic means to protect endogenous enkephalins and thereby to reinforce their physiological actions.

The discovery and characterization of enkephalinase (EC 3.4.24.11.) in brain by B. Malfroy in the laboratory of Pr JC Schwartz (INSERM, U 109, Paris) [1] and the demonstration that this peptidase was responsible for the inactivation of endogenous enkephalins [2] paved the way for the novel therapeutic approach. The same team, in collaboration with B.P. Roques (INSERM) and JM Lecomte (BIOPROJET), undertook the rational design of selective inhibitors of this enzyme.

Enkephalinase is present all along the gastrointestinal tract and its inhibition delays the inactivation of exogenous enkephalins in the guinea pig ileum.

[R] and [R,S]acetorphan selectively inhibit enkephalinase [EC 3.4.24.11.]. However, the selectivity of the inhibition is better for the [R] than for the [R,S]acetorphan with respective ratio of $ID_{50}\ \frac{\text{ACE inhibition}}{\text{Enkephalinase inhibition}}$ of 3000 and 100.

Enkephalins are endogenous opioids which elicit intestinal antisecretory activity without affecting intestinal transit [3]. [R] and [R,S]acetorphan by the inhibition of enkephalinase reinforce the physiological activity of endogenous enkephalins and show intestinal antisecretory activity [4-6] without affecting intestinal transit.

Toxicity studies have been carried out both with [R] and [R,S]acetorphan.

A complete toxicological evaluation has been performed with [R,S]acetorphan including :

- acute studies (rat, mouse, monkey, rabbit and dog),
- subacute studies (rat, dog, macacus monkey)

and chronic studies (6 months in rat and dog, 12 months in monkey).

A bridging program was performed with [R] and [R,S]acetorphan including :

- Acute studies (rat and mouse),
- Sub-acute studies (13 week in monkey, the most sensitive species to [R,S]acetorphan)

This bridging program evidences the same toxicological profile for [R,S]acetorphan and [R]Acetorphan and allows to use all the data collected with the racemate for the [R]Acetorphan.

Teratogenicity (rat, rabbit) and mutagenicity have been investigated with [R,S]acetorphan, no toxic effect was reported.

Studies of pharmacodependance and tolerance (rat, monkey) demonstrated the absence of addictive properties of [R,S]acetorphan.

[R] and [R,S]acetorphan, prodrugs, are rapidly metabolized in their respective active metabolites.

Pharmacokinetic profiles were studied using the level of plasmatic enkephalinase activity as measurement. After one single oral administration of [R,S]acetorphan, the maximal inhibition (C_{max}) of enkephalinase was of 80 %. The peak of inhibition (T_{max}) is observed between 1 and 2 hours after the administration of the drug. The biological half-life is three hours. With 300 mg, enkephalinase is significantly inhibited for 8 hours. Kinetic parameters are identical after a single and after multiple administration.

Kinetics is not modified in elderly but the intake of a meal just after a capsule intake delays the T_{max} of 1 hour without any modification of the C_{max} and the AUC of the enkephalinase inhibition.

[R,S]acetorphan is distributed in peripheral organs and does not cross the blood brain barrier after oral administration.

Pharmalogical studies in animals have demonstrated the antisecretory activity of [R] and [R,S]acetorphan on several experimental models of diarrhea.

The intestinal antisecretory activity was demonstrated :

- on a cholera-toxin model in dog [4] and in rat [5], and, after castor-oil administration in rat for [R,S]acetorphan [6],

- on a cholera-toxin model in rat for [R]Acetorphan [5].

This intestinal antisecretory effects is observed only in case of hypersecretion and not in the basal state, and is inhibited by naloxone demonstrating thus the enkephalins pathway of acetorphan activity [4].

The respective intestinal antisecretory effects of [R] and [R,S]acetorphan were compared in rat after cholera toxin [5]. They were of the same magnitude.

This antisecretory activity is also observed on gastric acid secretion [7,8] and on experimental cholecystitis in animals [9].

[R,S]acetorphan does not modify intestinal transit both in mice and in rat whereas on the same model, loperamide significantly lowers it [6].

Acetorphan does not modify psychometric tests and vigilance in healthy volunteers [10].

All these studies also demonstrated the lack of extra-digestive effect after oral administration.

Pharmacodynamic studies in healthy volunteers bring to the fore the same profile of activity.

An intestinal antisecretory activity of [R,S]acetorphan demonstrated both against cholera-toxin intestinal hypersecretion [11] and castor-oil induced diarrhea [12].

[R,S]acetorphan does not modify gastric emptying [13,14], oro-caecal and colonic transit times [15] in healthy volunteers.

[R,S]acetorphan is distributed in peripheral organs and does not cross the blood brain barrier after oral administration.

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[R,S]acetorphan does not modify gastric emptying [13,14], oro-caecal and colonic transit times [15] in healthy volunteers.

The tolerance of [R,S]acetorphan has been evaluated on 2 364 subjects treated at doses between 1.5 and 30 mg/kg and for a duration of treatment up to 3 months. The tolerance was good. The biological tolerance on more than 500 subjects was also good without any sign of toxicity of [R,S]acetorphan. The post marketing survey was good on 1 500 000 patients already treated.

[R,S]acetorphan obtained in 1992 the approval from the French Health Minister and has been launched for the treatment of acute diarrhea since March 1993.

[R]Acetorphan has already been given :

- in 9 healthy volunteers at 30 mg (single dose),
- in 6 healthy volunteers at 50 mg (single dose),
- in 4 healthy volunteers at 75 mg (single dose)
- in 25 patients at 100 mg t.i.d. during 6 weeks.

For these above studies, the tolerance was very good.

24 healthy volunteers received three doses (37.5, 75.0 and 150 mg) consecutively in single administration. Some minor and well tolerated adverse events were noted but not drug-related.

177 patients received 300 mg/day. It has not been noticed any serious adverse event or adverse event leading to study discontinuation. Only one adverse event related to the treatment (Abdominal pain and nausea quoted mild) has been reported.

12 patients received 300 mg/day during 7 days (Data analysis is in progress, at the time being, no serious adverse event has been noted).

[R,S]acetorphan : pediatric formulation

The pharmacokinetic profile, the efficacy and the clinical tolerance of acetorphan were evaluated in 3 studies on 280 children suffering from acute diarrhea treated at a mean dose of 1.5 mg/kg t.i.d.

These studies demonstrate :

- a pharmacokinetic profile similar to adults,
- an efficacy of acetorphan on stool weight in comparison to placebo,
- an efficacy similar to the loperamide one with a better tolerance (fewer constipation and associated treatments under acetorphan),
- a good tolerance whatever the age of the patients below or older than 2 years.

[1] MALFROY B, SWERTS JP, GUYON DA, ROQUES BP, SCHWARTZ JC. High affi enkephalin degrading peptidase in brain is increased after morphine. Nature, 1978, 176, 5 526.

[2] PATEY G, DE LA BAUME S, SCHWARTZ JC, GROS C, ROQUES BP, FOURNI ZALUSKI MC, SOROCA-LUCAS E. Selective protection of methionine enkephalin releas from brain slices by enkephalinase inhibition. Science, 1981, 21, 1153-1155.

[3] SHOOK J, LEMCKE P, GEHRIG C, HIRUBY J, BURKS T. Antidiarrheal properties supraspinal Mu and Delta and peripheral Mu, Delta and Kappa opioid receptors : inhibition diarrhea without constipation. J. Pharmacol. exp. Ther., 1989, 249, 83-89.

[4] PRIMI MP et Coll. Intestinal antisecretory activity of an enkephalinase inhibitor, Acetorphar in vivo demonstration in dogs. Gastroenterology, 1992, 102, A235.

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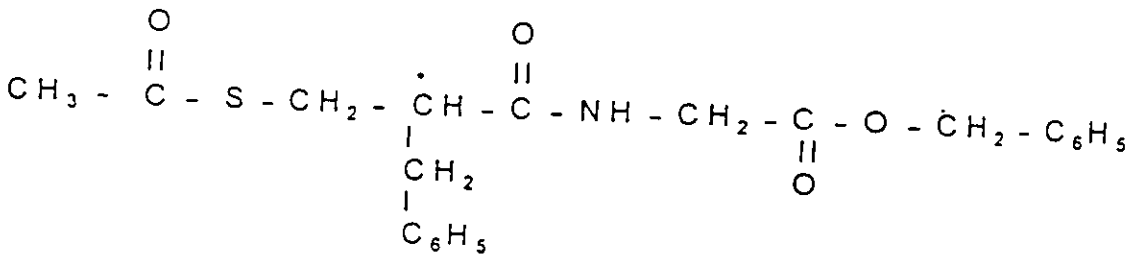
2. PHYSICOCHEMICAL CHARACTERISTICS

2.1 CHEMICAL NAME

N(R,S)-[(2-acetylthio)methyl]-1-oxo-3-phenyl propyl benzyl ester

2.2 DEVELOPED FORMULA



* Chiral centre

2.3 MOLECULAR FORMULA : $C_{21}H_{23}NO_4S$

2.4 CODE AND DENOMINATION

BP 0.52 : [R,S]Acetorphan
BP 1.01 : [R]Acetorphan

2.5 MOLECULAR WEIGHT : 385

2.6 SOLUBILITY

According to the European Pharmacopeia (2nd edition, IV-9-1991), [R,S]acetorphan solubilities are the following :

- very soluble in chloroform,
- freely soluble in acetone, methanol, sulfolane, toluene and ethanol,
- soluble in ethylether,
- practically insoluble in hexane and water.

2.7 DRUG PRODUCT

[R,S]acetorphan : 100 mg capsules, granulated powder
[R]acetorphan : 37.5 mg, 75 mg, 150 mg and 225 mg tablets

2.8 UNIT FORMULA

[R,S]acetorphan capsules

. [R,S]acetorphan	100
. Lactose	41
. Starch 1500	75
. Magnesium stearate	7
. Aerosil	2
	225 mg

for a size 2 capsule : turquoise blue/blue.

[R,S]acetorphan powder (one dose of administration = a child weighing 4 kg)

. [R,S]acetorphan	6.00
. Eudragit	0.90
. Saccharose	579.90
. Flavour	12.00
. Aerosil	1.20
	600 mg

[R]acetorphan

[R]acetorphan weight (mg)	37.50	75.0	150	225.0
- Lactose	27.75	55.5	111	166.5
- Corn starch	9	18	36	54
- Calcique CMC	6.55	13.1	26.2	39.3
- H P C	1.5	3	6	9
- Microcristalline cellulosis	10.3	20.6	41.2	61.8
- Magnesium stearate	0.4	0.8	1.6	2.4
- Theoretical weight uncoated tablet	93	186	372	558
- Film coating	5	5	5	5
- Theoretical weight Coated tablet	98	191	377	563

2.9 STABILITY

[R,S]acetorphan

a) Drug substance : [R,S]acetorphan

No alteration of [R,S]acetorphan occured under the action of light, humidity and temperature (20°, 37°, 50° C). The assay was not modified over a storage of 7 years.

b) Drug product

No alteration of the capsule or the capsule content was evidenced after 3 years of storage at ambient temperature and humidity or 1 year of storage at 37° C, 80 % of relative humidity.

2.10STORAGE CONDITION

No specific storage precautions are necessary.

2.11SHELF-LIFE

[R,S]acetorphan : 3 years
[R]acetorphan : 1 year

3. TOXICOLOGICAL STUDIES

All studies were done according to GMP and international guidelines.

3.1 SINGLE DOSE TOXICITY

Single dose toxicological studies have been performed in two species : Swiss mice and Sprague Dawley OFA rat with [R,S]acetorphan and [R]acetorphan given orally [1, 2].

No death or clinical abnormality was registered with doses of [R,S]acetorphan and [R]acetorphan up to 5 000 mg/kg.

3.2 SUBACUTE TOXICITY (4 weeks)

Subacute toxicity studies have been performed in three species : rat [3], dog [4] with [R,S]acetorphan and monkey [5, 6] with [R] and [R,S]acetorphan.

- Sprague Dawley rats

No clinical abnormality was observed with the 100 and 500 mg/kg/day doses.
At 2 500 mg/kg/day (highest dose), a slowing down of bodyweight gain and a polyuria with a polydypsia were observed.

- Beagle dog

The 50 mg/kg/day dose was very well tolerated.

For the higher doses :

- Transient episodes of diarrhea were observed at 200 and 800 mg/kg/day.

- Transient vomiting episodes at 800 mg/kg/day.

A non hemolytic anemia in 2/6 animals at 200 mg/kg/day and in 3/6 animals at 800 mg/kg/day.

- Cynomolgus monkey

First study

[R,S]acetorphan was administered at the dose levels of 50, 250 and 1 250 mg/kg/day.

The following abnormalities were observed :

- transient vomiting and diarrheic episodes at 250 and 1 250 mg/kg/day,
- anemia in one animal at 1 250 mg/kg/day.

In conclusion, the lowest toxicological dose in the most sensitive species is 40 fold higher than the therapeutic dose.

Second study

A bridging 13 week toxocological study was conducted in monkey, the most sensitive species to [R,S]acetorphan.

[R,S]acetorphan was administered at the dose levels of 50, 110 and 240 mg/kg. No death was observed, the toxicological profiles of [R]acetorphan and [R,S]acetorphan were similar with the following abnormalities in both groups : vomiting, diarrheic episodes and buccal lesions.

In conclusion, this study shows a similar toxicological profile for [R]acetorphan and [R,S]acetorphan and allows to use all the toxicological data on [R,S]acetorphan for [R]acetorphan.

3 CHRONIC TOXICITY

Three chronic studies have been performed in three different species : rodent (rat, 6 months) [7] and two non rodents (dog, 6 months [8] - monkey, 12 months [9]).

The following doses have been given :

. Sprague Dawley rat : 0 - 100 - 300 - 1 000 mg/kg/day
20 to 30 animals/dose/sex

. Beagle dog : 200 mg/kg/day - 4 animals per sex

. Cynomolgus monkey : 0 - 50 - 150 - 500 mg/kg/day
3 - 5 animals/dose/sex

. Rat

No death related to [R,S]acetorphan was observed.

At 100 mg/kg, only a very slight decrease (- 4 %) in male bodyweight gain was noted.

At 300 mg/kg, the following changes were noted :

- a very slight decrease in bodyweight gain in the males,
- slight changes in urine analysis parameters : decrease of pH, presence of ketones,
- slight changes in organ weights : decrease in heart weight, increase in liver weight.

In the animals receiving 1 000 mg/kg/day, the following changes were observed :

- minimal clinical signs : salivation and very slight decrease in bodyweight gain in the males,
- changes in blood biochemistry parameters, particularly associated with lipid metabolism : decrease in total lipids level,
- changes in urine analysis parameters : decrease of pH, presence of a high quantity of ketones,
- slight changes in organ weight : decrease in heart weight, decrease in liver and kidney weights,
- the onset of hepatic steatosis in a few animals after 26 weeks dosing.

. Dog

This study was designed to check a possible haematological toxicology besides the usual parameters. The following of the haematological parameters was very closed : hemogram before treatment and each fournight during treatment, myelogram before treatment and every 6 weeks during treatment.

A normochromic, macrocytic, only slightly regenerative and non haemolytic anemia was noted after 6 weeks dosing in one male and one female. In the female, these changes were spontaneously reversible from week 8 of treatment onwards.

In the male, the severity of the anemia increased suddenly and the animal was killed in a moribund state at week 14.

There were no pathological changes in the bone marrow and no histopathological changes in any animals.

This haematological change, probably related to an immunological disorder remained isolated as no other of the 7 animals showed evidence of the same type of finding during the course of the second half of the treatment period.

The haematological toxicity of captopril (IACE) was studied following daily oral administration to two Beagle dogs at a dose level of 600 mg/kg/day for 32 consecutive days. At the end of treatment, non-regenerative anemia and thrombocytopenia were observed in the two animals. These results are in favour of a particular sensitivity of dog to the ACE inhibition which is also observed with the highest doses of acetorphan.

- Cynomolgus Monkey

The 50 and 150 mg doses were well tolerated.

Six out of ten high dose group animals died or were killed between weeks 8 and 17 of the treatment period. This mortality was attributable to infections differing in nature from one animal to another one.

Following the observation of infectious conditions in high dose group, an evaluation of the immune function was performed in the treated animals which included a specific evaluation of blood cells involved in cell mediated immunity.

Furthermore, antibody-response to antitetanic vaccine was evaluated.
No modification of the immunologically competent cells was observed and all the animals responded normally to challenge with tetanic antigen except one male of high dose group which also had at the end of the treatment period an immune deficiency of auto-immune type.

Auto-antibodies were also present in one female of high dose group.

Complementary investigations have been performed to study more deeply t infections noticed on the 12 month study in monkey :

- immunotoxicological exploration was performed on different models in mice [1(testing respectively the reaction after an experimental infection, the humora immunity after treatment with [R,S]acetorphan (1 000 mg/kg/day). N abnormality was observed in the three tests suggesting that the infections observe in monkey were not related to an immunodepressive action of acetorphan.

3.4 REPRODUCTION STUDIES

No toxicity was observed on fertility and the reproductive performances [11]. No embryotoxicity or teratogenic activity was observed [12,13]. No peri or post-natal toxicity [14] was observed. The highest dose tested was 1 000 mg/kg/day of [R,S]acetorphan.

3.5 SPECIFIC TOXICOLOGICAL STUDIES

. Mutagenic potential

No mutagenic or clastogenic potential was observed on the three in vitro [15-17] tests with or without metabolic activation and on the in vivo [18] test realized in comparison with a positive control.

. Dependance risk study

Physical dependence was studied in rat and in rhesus monkey [19]. In both species, acetorphan does not prevent the withdrawal syndrom in animals accustomed to morphine. The continuous injection with acetorphan in rat followed by stoppage provokes no withdrawal syndrome. Psychic dependance was studied in the rhesus monkey accustomed to cocaïne. No significant effect of acetorphan was observed.

. Central nervous system

Effect on motor activity

No effect of acetorphan on motor activity was observed after oral administration.

Analgesic effect

No analgesic effect of acetorphan was observed after oral administration [20].

Cardiovascular effect

No modification of blood pressure and heart rate was observed in animal studies [21].

Drug interaction

Possible drug interactions were investigated in various studies :

- Duration of pentobarbital anesthesia in the mouse : [R,S] acetorphan does not alter duration of pentobarbital induced sleep [22]. Therefore it does not induce any enzymatic induction, contrarily to phenytoine, the reference subtance.
- Anti K-vitamine effect of acenocoumarol in the rat : oral administration of [R,S] acetorphan 3 or 10 mg/kg/day does not modify the antiblotting effect of acenocoumarol [23].
- Tolbutamide induced hypoglycemia in the rat : oral administration of [R,S] acetorphan 3 or 10 mg/kg.day does not modify mebeverine or trimebutine effect on intestinal transit [24].
- Protein binding

  BP 0.35 (the active metabolite) is strongly bound to plasma proteins (91 %) mostly to albumine. It does not alter protein binding of the tested drugs (warfarine, tolbutamide, niflumic acid, phenytoin, digitoxin) and vice versa [25].

Conclusion

Toxicological studies, after single or repeated administration, have shown the safety of the drug and the wide gap between the therapeutic and the toxic doses. [R,S] acetorphan is neither mutagenic, nor clastogenic and has no effect on reproductive functions. It does not interfere with the main body functions, is not an hepatic enzymatic inductor, does not modify anti K-vitamin and biguanide activity. It induces no dependance liability.

The acute and subacute toxicological studies show that the safety of [R] and [R,S]acetorphan are similar.

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[2] HAZLETON France. Acute oral toxicity to rats of BP 0.52 and its stereoisomers BP 1.0 BP 1.01. 1992. Rapport interne.

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[4] HAZLETON France. Toxicité de 4 semaines par voie orale chez le chien Beagle. Do A.M.M. TIORFAN®, volume 7, rapport 506250.

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4. ANIMAL PHARMACOLOGY

The multiple studies performed after oral administration with [R,S]acetorphan have shown its elective activity on the digestive tract without any significant effect on other organs.

4.1 INTESTINAL ANTISECRETORY ACTIVITY

The intestinal antisecretory activity of [R,S]acetorphan has been demonstrated in two models : cholera toxin in dogs and castor-oil diarrhea in rodents.

- Cholera toxin induced diarrhea in dog [1]

[R,S]acetorphan administered orally has been investigated in conscious dogs on an isolated jejunal loop (Thiry-Vella method) in basal conditions or after cholera toxin-induced hypersecretion.

A balanced electrolyte solution (Na^+ = 140, K^+ = 5, Cl^- = 110 ; HCO_3 = 35 mEq/l ; glucose = 26 mM), containing [^{14}C]. PEG 4000 as a non-absorbable marker was infused into the loop at a rate of 2 ml/min. The effluent was collected in 20 min samples which were analyzed for PEG concentration.
Na^+ and K^+ were measured by flame photometry.

Intestinal hypersecretion was provoked by addition of cholera toxin (0.4 µg/ml) in tyrode solution at t = 0.
After 2 hours, equilibration was obtained and the infusion was maintained for 2 additional hours. Then tyrode solution containing ^{14}C P.E.G. infusion was continued for 4 hours. So, the whole experiment lasted for 8 hours, maximal secretion being observed 3-4 hours after cholera toxin perfusion.

Two series of 3 dogs were used and two experiments were performed on each animal.

First series of dogs

1st trial : control according to the schedule shown in figure 1 (next page).

2nd trial : administration of [R,S]acetorphan (10 mg/kg per os), 1 hour before addition of cholera toxin in the perfusion (t = - 1 h).

Basal absorption was measured during steady state infusion in samples collected 100, 120 and 140 min after the onset of infusion.

Second series of dogs

[R,S]acetorphan was orally administered (10 mg/kg) and its effect was antagonized by administration of either naloxone (0.1 mg/kg) or phentolamine (0.2 mg/kg) 10 min before [R,S]acetorphan. Three experiments were performed on each animal. All the results were analyzed by a Wilcoxon test.
Calculations are summarized in figure 1 (next page).

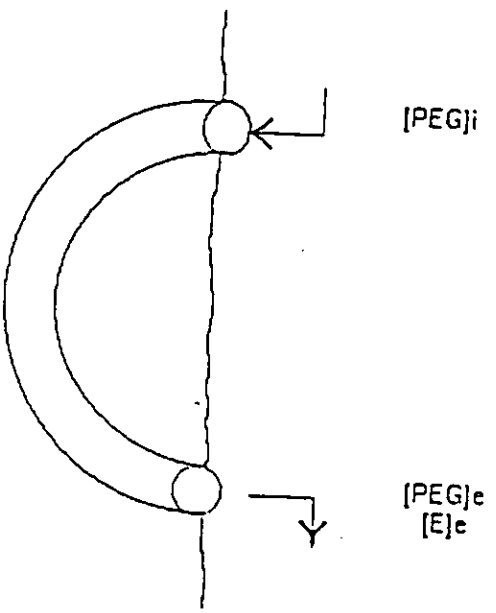
STUDY OF THE DIGESTIVE MOTILITY AND ANTISECRETORY PROPERTIES IN DOGS

Figure 1

Infusion rate IR = 2 ml.min^{-1}

Tyrode : Na " 3.22 g.l^{-1}
K " 0.195 g.l^{-1}
Cl^- " 3.9 g.l^{-1}
HCO_3^- 2.2 g.l^{-1}

[E]^1



Summary of calculations

Net flux of H_2O (g.l^{-1}) per min = IR - IR x $\frac{[PEG]\ i\ \text{infusate}}{[PEG]\ e\ \text{effluent}}$

Net flux of Na (mEq.l^{-1}) per min = [E]i. IR - [E] e.IR . $\frac{[PEG]\ i}{[PEG]\ e}$

STUDY OF THE INTESTINAL ANTISECRETORY PROPERTIES IN DOGS

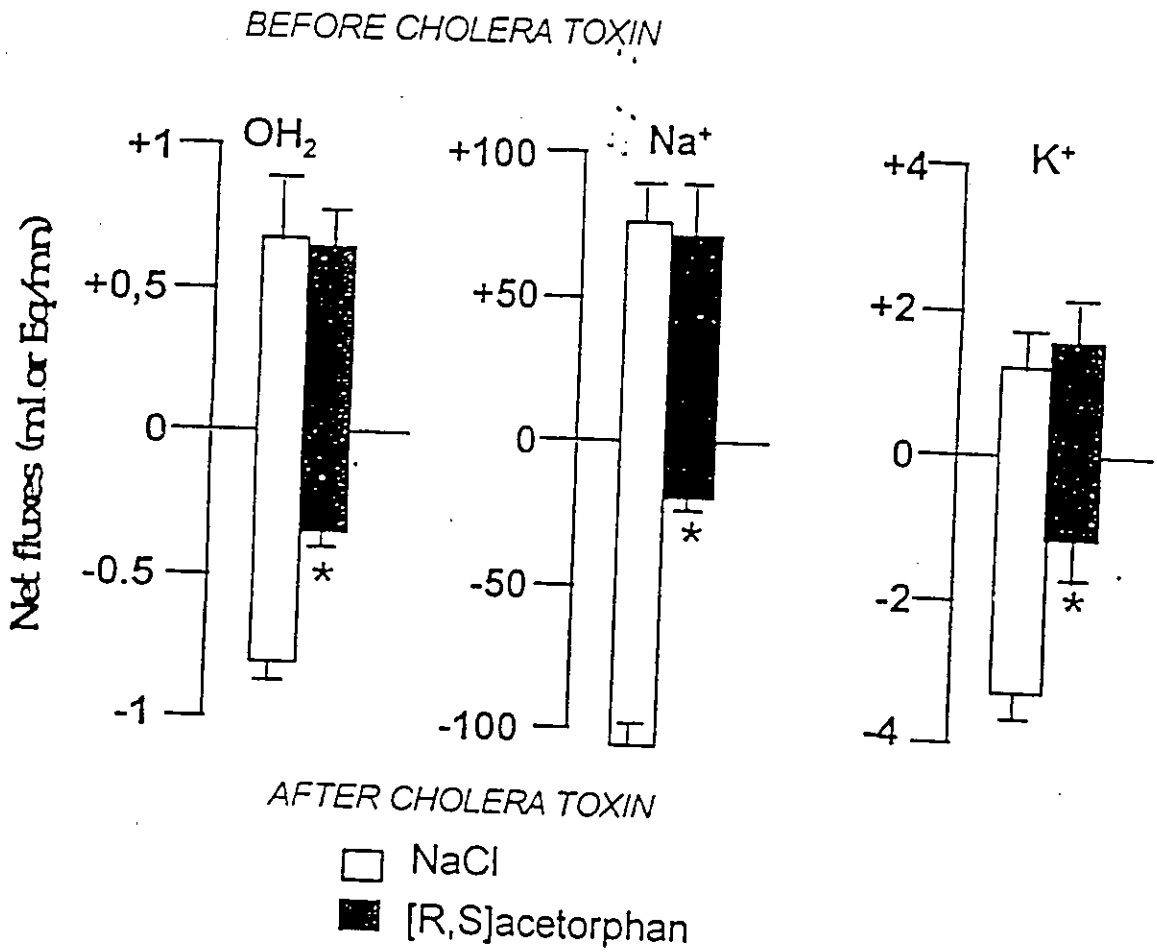


Figure 2 : Effects of [R,S]acetorphan (10 mg/kg. p.o.) administered orally on the rate of basal absorption of water, Na^+ and K^+ and after cholera toxin in a jejunal loop in conscious dogs.
Means ± S.E., n = 9 - (+) denotes absorption ; (-) denotes secretion
* $P < 0.05$ W test

sued from L. Bueno, B. Primi et al (1987)

ed August 17, 1995

After cholera toxin, net flux of water was statistically decreased (- 0.73 ± 0.14 ml/min in control as compared to - 0.38 ± 0.07 ml/min in treated dogs).

Secretion of electrolytes was considerably reduced after [R,S]acetorphan as compared to cholera toxin control.

Na^+ = - 125 ± 11 µEq/min in control vs - 14 ± 16 in treated group.

K^+ = - 3.4 ± 0.6 µEq/min in control vs - 1.6 ± 0.8 in treated group.

In a second series of experiments, similar effects were evidenced. It was found that these effects were antagonized by naloxone but failed to be antagonized by phentolamine.

In conclusion, this study clearly shows that oral [R,S]acetorphan (10 mg/kg) has an **intestinal antisecretory effect when secretion is stimulated by cholera toxin.**

This effect seems to involve endogenous opioids as shown by the naloxone antagonism.

Cholera toxin induced diarrhea in rats [2]

[R] and [R,S]acetorphan administered intraduodenally at 2 and 10 mg/kg have been investigated in anaesthesiated rat after cholera toxin-induced hypersecretion.

Animal preparation

After laparotomy under urethane (3 g/kg. i.p.) anaesthesia, a 15 cm segment of the proximal jejunum (5 to 20 cm from the ligament of Treiz) was isolated, rinced with a Tyrode solution at 37° C, cannulated at the two extremities and replaced in the abdominal cavity. During all the experiments, the animals were maintained on a hot plate to avoid hypothermia.

Water flux measurement

A Tyrode solution containing 2 g/l of cold polyethylene glycol 4000 (PEG 4000) added with 5 mCi/l of ^{14}C radiolabelled PEG 4000 was infused in the jejunal loop at a constant rate of 6 ml/hr. The effluent was collected at 15 min intervals on a fraction collector. Radioactivity in the effluents and the infused Tyrode solution was determined by liquid scintillometry using a β-counter (Beta V, Kontron, Basle, Switzerland).

Net water flux by 15 min periods was calculated as follows :

$$\text{- Net flux } (\mu l/cm/h) = (1 - DPMs/DPMc) \times P/L$$

where : DPMs = ^{14}C radioactivity in the infused solution
DPMc = ^{14}C radioactivity in 15 min effluents
P = infusion rate (6000 µl/h)
L = length of the intestinal segment (cm)

Intestinal hypersecretion was provoked by addition of cholera toxin (1 µg/ml) in Tyrode solution :

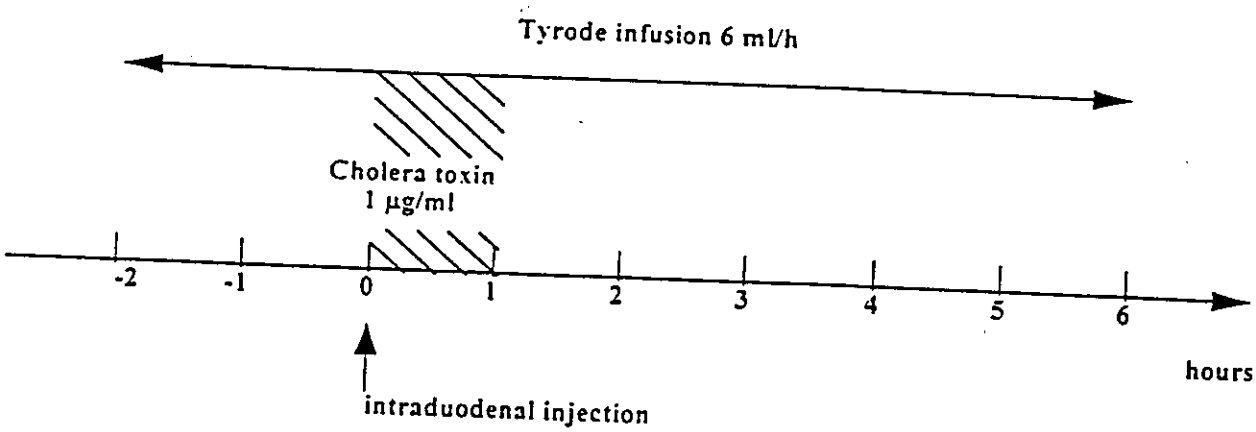


Table 1 : RESULTS

TIME	CONTROL	[R,S]acetorphan		[R]acetorphan	
		2 mg/kg	10 mg/kg	2 mg/kg	10 mg/kg
T0	148 ± 21	134 ± 11	146 ± 21	164 ± 31	126 ± 19
T1	153 ± 20	153 ± 18	158 ± 20	144 ± 30	159 ± 36
T2	165 ± 17	134 ± 19	161 ± 25	135 ± 26	142 ± 37
T3	-165 ± 37*	166 ± 45@	160 ± 38@	154 ± 25@	158 ± 20@
T4	-340 ± 52*	122 ± 25@	84 ± 25*@	190 ± 19@	150 ± 28@
T5	-401 ± 38*	79 ± 11*@	57 ± 8*@	156 ± 24@	97 ± 30@
T6	-442 ± 78*	59 ± 28*@	62 ± 13*@	81 ± 12*@	59 ± 14*@

* Significantly different from T0 [$P < 0,05$, $n = 6$]

@ Significantly different from Control group at the corresponding time [$P < 0.05$, $n = 6$]

Cholera toxin reverses water fluxes into a net secretion from the 3rd hour until the end of the experiment in the control group.

[R] and [R,S]acetorphan at doses of 2 and 10 mg/kg totally abolish the jejunal hypersecretion with no statistically significant difference between the 2 products even if [R]acetorphan is slightly more effective than the [R,S]acetorphan.

Castor-oil induced diarrhea in rodents [3]

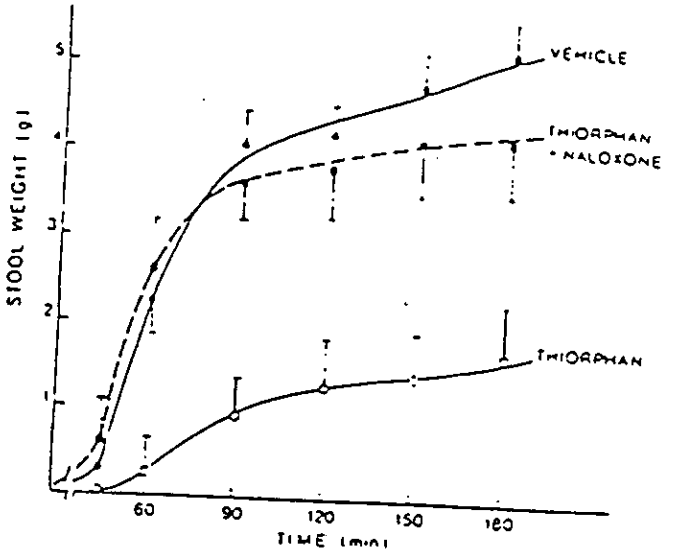
The test was performed on male Wistar rats (180 - 200 g) and Swiss mice (18 - 20 g) housed in makrolon cages at an ambient temperature of 21° C having a free access to standard rodent diet and water. They were deprived of food but not water during the 18 - 24 hr period preceding the experiments.

The castor oil test described by Niemegeers et al (1972) was used with minor modifications. Following the various treatments, starved rats received a standard dose of castor oil administered orally by gavage (1.0 ml) and were individually caged. The onset of diarrhea was noted during a 4 hour observation period. Stools were collected on pieces of absorbent paper placed beneath the grilled floor of the cage and weighed at 15 min intervals from 45 min after the castor oil challenge (time at which diarrhea had not yet started).

Diarrhea was evaluated as the cumulative stool weight during the experimental period.

Thiorphan, the active metablite of [R,S]acetorphan, (40 mg/kg, i.v.) significantly ($P < 0.01$) protected rats against the castor oil-induced diarrhea, an effect particularly marked during the first 90 min period following the castor oil challenge but still apparent after 3 hours (Figure 3) and even after 4 hour time at which stool weights were 2.1 ± 0.5 g as compared to 5.9 ± 0.5 g in controls. Naloxone (2 x 2 mg/kg. s.c.), an opiate receptor antagonist, completely reversed the antidiarrheal effect of thiorphan since cumulative stool weights in rats receiving both thiorphan and naloxone did not significantly differ from those of control animals at any time.

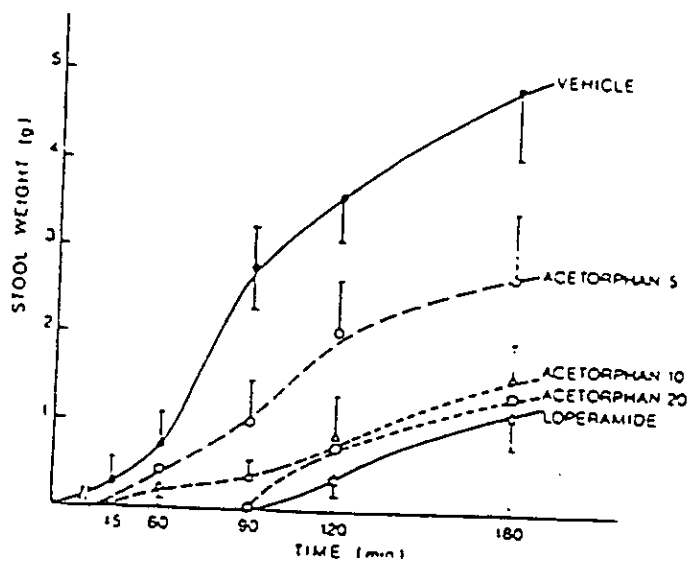
Figure 3



Effects of thiorphan and naloxone on castor oil-induced diarrhea in rats. Rats received thiorphan (40 mg/kg i.v.) 30 min before the administration of castor oil. Naloxone (2×2 mg/kg s.c.) was administered 10 min before and 25 min after thiorphan. The values represent the cumulative stool weights. Means ± S.E.M. of 8-16 experiments.

[R,S]acetorphan (5 mg/kg, i.v.) reduced by about 50 % the cumulated stool weight during the 3 hr experimental period (Figure 4) and the effect was still significan ($P < 0.05$) after 4 hour time at which stool weights were 3.2 ± 0.7 g as compared to 5.7 ± 0.7 g in controls. The effect was even more marked at 10 and 20 mg/kg i.v. At 10 mg/kg, stool weights were still significantly ($P < 0.01$) reduced after 8 hours (3.0 ± 0.5 g as compared to 6.3 ± 0.8 g in controls). At 20 mg/kg, the diarrhea was delayed up to about 3 hours and the effect did not significantly differ from that observed with loperamide (2 mg/kg, p.o.). The effects of [R,S]acetorphan (10 mg/kg, i.v.) on either onset of diarrhea or stool weights were prevented in rats treated with naloxone (2 x 2 mg/kg, s.c.).

Figure 4



Effects of acetorphan and loperamide on castor oil-induced diarrhea in rats. Rats received acetorphan (5, 10 or 20 mg/kg i.v.) or loperamide (2 mg/kg p.o.) 30 min before the administration of castor oil. The values represent the cumulative stool weights. Means ± S.E.M. of 7-12 experiments.

[R,S]acetorphan (10 mg/kg, p.o.) significantly delayed the onset of diarrhea, approximately to the same extent as at higher dosages. A significant antidiarrheal effect, corresponding to about 50 % reduction in cumulated stool weights during the 90 min experimental period, occured as a result of oral administration of [R,S]acetorphan (20 mg/kg) and this was even slightly more marked at 40 mg/kg. The antidiarrheal activity of [R,S]acetorphan (20 mg/kg, p.o.) was prevented by naloxone (s.c.).
In contrast, [R,S]acetorphan (0.1 mg, i.c.v.) did not significantly modify stool weights or onset of diarrhea as compared to controls (Table 2).

Table 2

TREATMENTS	Onset of diarrhea [min]	Stool weight [g]
Vehicle	70 ± 4	3.7 ± 0.5
Thiorphan (7 mg/kg, i.v.)	67 ± 6 N.S.	3.9 ± 1.1 N.S.
Thiorphan (20 mg/kg, i.v.)	163 ± 40 a	1.4 ± 0.6 a
Thiorphan (40 mg/kg, i.v.)	179 ± 45 b	0.8 ± 0.3 b
Vehicle	73 ± 5	4.2 ± 0.5
Thiorphan (40 mg/kg i.v.)	213 ± 52	1.0 ± 0.4 b
Naloxone (2 x 2 mg/kg, s.c.)	90 ± 15 N.S.	2.8 ± 0.7 N.S.
Thiorphan (40 mg/kg, i.v.) + naloxone (2 x 2 mg/kg s.c.)	81 ± 11 N.S.	4.4 ± 0.6 N.S.
Vehicle	78 ± 11	4.5 ± 0.6
Thiorphan (40 mg/kg i.v.)	194 ± 56 a	0.4 ± 0.4 b
Naloxone (2 x 2 mg/kg, s.c.)	81 ± 8 N.S.	2.9 ± 0.4 a
Thiorphan (40 mg/kg, i.v.) + naloxone (50 µg i.c.v.)	211 ± 38 b	0.7 ± 0.4 b.c
Vehicle	-	2.5 ± 0.6
Thiorphan (0.15 mg i.c.v.)	-	2.8 ± 0.7 N.S.
Vehicle	79 ± 13	2.1 ± 0.9
Thiorphan (20 mg/kg p.o.)	82 ± 9 N.S.	2.8 ± 0.8 N.S.

a : P < 0.05, b : P < 0.01, N.S. non significant as compared to the respective controls
c : non significant as compared to thiorphan alone
mean ± S.E.M.

In a more limited series of experiments (not shown), a significant (P < 0.001) inhibition of castor-oil induced diarrhea was observed in mice treated with [R,S]acetorphan (20 mg/kg, i.v.) or loperamide (0.5 mg/kg, i.v.).

Another test was performed on male Wistar rats (150-200 g). They were deprived of food but not water during the 24 hr period preceding the experiments.

The castor-oil test described by Niemegeers et al (1972) was also used with minor modifications. Thirty minutes after treatment adminsitration, a standard dose of castor-oil (1.0 ml) was administered orally by gavage. Two doses of [R] and [R,S]acetorphan were tested 1.5 and 7 mg/kg. The onset of diarrhea was noted during a 5 hour observation period. Stools were collected on pieces of adsorbent paper placed beneath the grilled floor of the cage and weighed at 45, 60, 90, 120, 150, 180, 240 and 300 minutes after castor-oil. Diarrhea was calculated on the cumulative stool weight.

Table 3 : RESULTS

	[R,S]acetorphan		[R]acetorphan	
	1.7 mg/kg	5 mg/kg	1.7 mg/kg	5 mg/kg
% OF DECREASE OF CUMULATED STOOL WEIGHT	36 %	40 %	41 %	49 %

The magnitude of the stool weight is identical for [R] and [R,S]acetorphan even if the results are slightly in favour of [R]acetorphan.

4.2 EFFECT OF [R,S]ACETORPHAN AND LOPERAMIDE ON GASTROINTESTINAL TRANSIT [3]

A charcoal suspension (10 % suspension of activated charcoal French Pharmacopeia in 5 % gum arabic) was given orally by gavage to starved rats (1 ml) or mice (0.2 ml). The animals were killed 20 min after receiving the charcoal and the intestines were carefully removed without stretching and placed lengthwise on moist paper. The distance travelled by the charcoal was evaluated and expressed as a percentage of the length of the small intestine (from the pyloric sphincter to the ileo-caecal junction) of each animal.

In rats, whereas loperamide (2 mg/kg p.o.) significantly reduced (by 27 %) the gastrointestinal transit as evaluated in the charcoal meal test, [R,S]acetorphan (40 mg/kg, p.o.) had no significant effect. (Table 4)

In addition, the gastrointestinal transit was evaluated in rats receiving castor oil 60 min before [R,S]acetorphan (40 mg/kg. p.o.) or loperamide (2 mg/kg. p.o.) and 80 min before the charcoal meal. The distance travelled (in percent of the total length of the small bowel) were significantly decreased (45 ± 5) in loperamide treated animals ($P < 0.01$) (mean ± S.E.M. of 6-7 experiments).

In mice, in which loperamide (0.5 mg/kg. i.v. or 10 mg/kg. p.o.) had a marked effect on a similar test, neither thiorphan nor [R.S]acetorphan (20 mg/kg. i.v.) did not inhibit the gastrointestinal transit. (Table 4)

Table 4

Effects of thiorphan, [R,S]acetorphan and loperamide on gastrointestinal transit as evaluated in the charcoal meal test in rats and mice

Means ± S.E.M. of 7-12 experiments

[The animals received [R,S]acetorphan, thiorphan or loperamide 20 min before the administration of the charcoal meal and were killed 20 min after the latter]

TREATMENTS	DISTANCE TRAVELLED [% of small bowel length]
[A] Rats	
Vehicle	67 ± 5
Acetorphan (40 mg/kg. p.o.)	64 ± 3 N.S
Loperamide (2 mg/kg. p.o.)	49 ± 2 a
[B] Mice	
Vehicle	57 ± 3
Thiorphan (20 mg/kg. i.v.)	61 ± 4 N.S
Acetorphan (20 mg/kg. i.v.)	57 ± 5 N.S
Loperamide (0.5 mg/kg. i.v.)	22 ± 3 b
Loperamide (0.5 mg/kg. p.o.)	61 ± 2 N.S
Loperamide (10 mg/kg. p.o.)	33 ± 3 b

a $P < 0.05$. b $P < 0.001$

The effects of [R,S]acetorphan (40 mg/kg, p.o.) on gastrointestinal transit along the large bowel were also assessed in groups of 10 mice receiving the drug 60 min after castor oi and 20 min after the charcoal meal and which were killed 150 min after this meal. The distance travelled, expressed as a percent of total length of the large bowel, was 33 ± 13 % as compared to 38 ± 9 % in controls.

In rats, a similar study performed on a smaller group of animals (n = 3) killed 240 min after the charcoal meal, showed that [R,S]acetorphan (40 mg/kg, p.o.) did not significantly modify the distance travelled along the large bowel (82 ± 10 % as compared to 88 ± 8 % in controls).

4.3 OTHER ANTISECRETORY ACTIVITIES

- **Effect of [R,S]acetorphan on mucosal gallbladder fluid secretion in experimental cholecystitis [4]**

The aim of this study was to characterize the effects of [R,S]acetorphan on the function of the inflamed feline gallbladder in comparison with control animals. As for the intestinal secretion, [R,S]acetorphan does not modify fluid secretion in the control state and significantly inhibits fluid secretion of an inflamed gallbladder.

- **Effect of [R,S]acetorphan on gastric acid secretion [5-6]**

A gastric antisecretory activity of [R,S]acetorphan was evinced in two species : rat and dog and both in the basal state or after stimulation by histamine, pentagastrin and 2 deoxy-glucose.

In conclusion, [R,S]acetorphan exerts an intestinal antisecretory activity only in case of hypersecretion with any effect on intestinal transit. This effect is mediated through enkephalins and mimics the effect of the agonist of the S receptors.

- **Effect of [R,S]acetorphan on gastrointestinal motility**

Besides their antisecretory activity, endogenous enkephalins are also involved in the physiological control of gastrointestinal motility. This aspect has also been studied with [R,S]acetorphan.

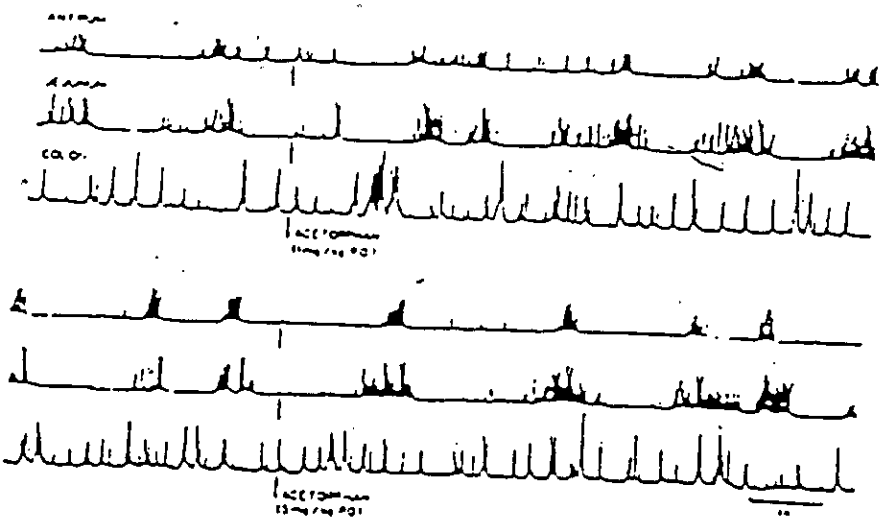
- Comparative study of the effect of [R] and [R,S]acetorphan on gastrointestinal motility in dog [7]

  The effect of [R] and [R,S]acetorphan (1 and 10 mg/kg) on gastrointestinal and colonic motility was studied both in fasted and fed dogs using chronically implanted strain gauges.

  [R] and [R,S]acetorphan induced the same effects on gastrointestinal and colonic motillity in fasted and fed dogs at equivalent doses.

In either fasted or fed dogs, [R] and [R,S]acetorphan at 1 and 10 mg/kg increased jeunal motility. [R] and [R,S]acetorphan did not modify fasted colonic motility. They increased fed colonic motility only after 10 mg/kg.

Figure 5



Effect of [R,S]acetorphan on colonic motility in cat [8]

The aim of this study was to look at the effect of BP 0.35, the active metabolite of [R,S]acetorphan, on colonic myoelectrical activity in cat.
The type of activity observed was different depending on the electric underlying activity of the colon. BP 0.35 stimulates the colon in the basal state. on the opposite, BP 0.35 inhibits the increase of motility induced by a parasympathic stimulation.

Effect of [R,S]acetorphan on colonic motility in rat [9]

The aim of this study was to assess the effect of [R,S]acetorphan (20 mg/kg x 2 subcutaneously) on colonic motility in rat using electromyographical recording.

[R,S]acetorphan does not modify the myoelectric index and increases the number of propagated waves and the percentage of the waves propagated on the entire colon.

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5. PHARMACOKINETICS

5.1 ANIMAL PHARMACOKINETIC STUDIES

. ^{14}C-[R,S]acetorphan : absorption, distribution, metabolism and excretion following a single oral dose to pigmented and non pigmented rats [1]

Assessed from urinary and pulmonary eliminations of radioactivity and distribution parameters, absorption of ^{14}C-[R,S]acetorphan is at least 61 % of the administered dose. Digestive tract, carcass as well as organs in charge of elimination (liver, kidney, lungs) contain the majority of radioactivity however always less than 1 % of the administered dose. No radioactivity is contained in CNS. No difference in the distribution of the radioactivity has been notified between pigmented or non-pigmented rats in eyes and skin where levels are less than 0.01 % of the administered dose. [R,S]acetorphan has no special affinity for melanin.

Plasma concentrations of [R,S]acetorphan and BP 0.35 are below the detection level (0.5 µg/ml) 2 and 4 hours after administration respectively. The major radioactive compound is metabolite M or methylthioether of thiorphan.

The total recovery of radioactivity is 92 % which is mainly eliminated in urine within the first 24 hours. 56.1 % of administered radioactivity is eliminated in urine, 26.9 % in feces.

. ^{14}C-[R,S]acetorphan : absorption, distribution, metabolism and excretion following oral and intraveinous administration to the Cynomolgus monkey [2]

Following a single administration of 30 mg ^{14}C-[R,S]acetorphan by intraveinous route, the peak of total radioactivity occurs within 15 to 30 minutes. Plasma concentrations decrease then rapidly in a bi-exponential curve. The "half-life" of radioactivity during the distribution/elimination phase is 0.8 hour.

After a 80 mg single oral dose of ^{14}C-[R,S]acetorphan, the plasmatic peak of radioactivity occurs 3 hours after administration.

[R,S]acetorphan is rapidly metabolized and only its active metabolite (BP 0.35) can be detected in plasma.

The tissue distribution is very low < 1 % of total radioactivity. The routes of elimination of radioactivity are : urine, feces and expired air.

5.2 HUMAN PHAROMACOKINETIC STUDIES

Study of the enkephalinase activity following a single oral dose of 30, 100 or 300 mg of [R,S]acetorphan in man [3]

After an oral administration of 30, 100 or 300 mg of [R,S]acetorphan, absorption is rapid, the peak of inhibition of the plasmatic enkephalinase activity occurs between 1 and 2 hours.

Inhibition is statistically significant compared to placebo at 1 hour for the 30 mg dose, between 0.5 and 8 hours for 100 mg dose and between 0.5 and 8 hours for 300 mg dose.

Areas Under the Curve (AUC) for [R,S]acetorphan 30 mg do not differ significantly from placebo.

AUC for [R,S]acetorphan 100 mg and 300 mg are significantly different from placebo without any significant difference between the 2 doses.

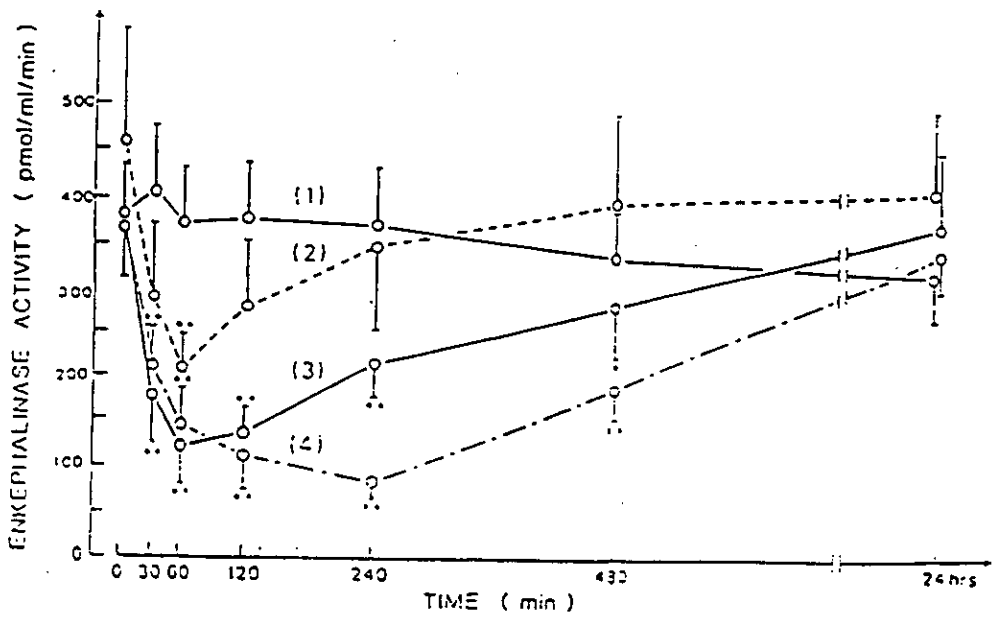

Figure 1 : Plasmatic enkephalinase activity after Placebo (1), [R,S]acetorphan 30 mg (2), [R,S]acetorphan 100 mg (3), [R,S]acetorphan 300 mg (4) administered by oral route to 8 subjects [* $P < 0.05$; ** $P < 0.01$, as compared to placebo]

- **Pharmacokinetic study of [R]acetorphan following a single oral dose of 50 mg in man [4]**

After an oral administration of 50 mg of [R]acetorphan :

- The maximal inhibition of enkephalinase was 80 % similar to what was observed with higher dose of [R,S]acetorphan,
- The Tmax was observed at 75 minutes.

In conclusion, a single dose of 50 mg of [R]acetorphan was enough to obtain the maximal inhibition of enkephalinase.

- **Pilot pharmacokinetic study of [R,S]acetorphan 100 mg capsule versus [R]acetorphan 75 mg tablet [5]**

A cross over pilot study was performed in 4 healthy volunteers who received one capsule of 100 mg [R,S]acetorphan, one tablet of 75 mg non micronized [R]acetorphan and one tablet of 75 mg micronized [R]acetorphan .

The pharmacokinetic profile was determined on enkephalinase inhibition and active metabolite ([R]and [R,S]Thiorphan) concentrations in plasma.

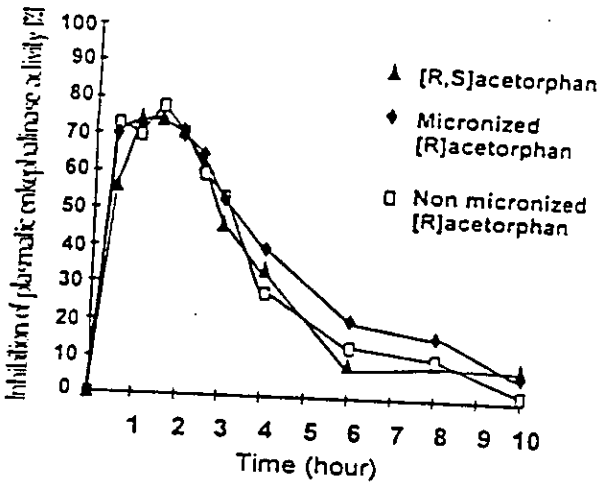

Figure 2 : Mean curves of plasmatic enkephalinase activity inhibition after 100 mg [R,S]acetorphan, 75 mg non micronized [R]acetorphan and 75 mg micronized [R]acetorphan

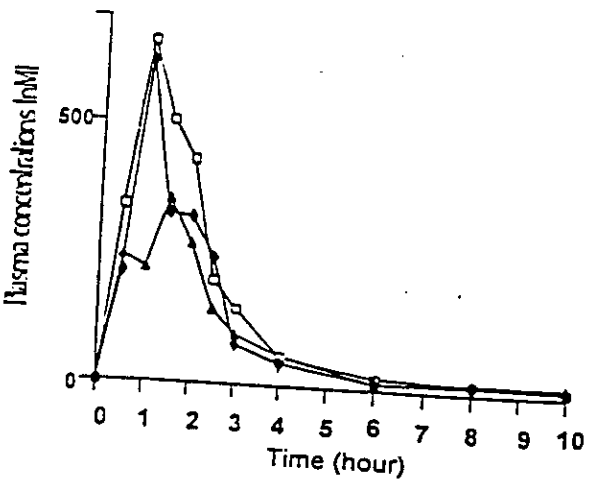

Figure 3 : Mean plasmatic concentrations of [R] and [R,S]-thiorphan after 100 mg [R,S]acetorphan, 75 mg non micronized [R]acetorphan and 75 mg micronized [R]acetorphan

The results observed in this pilot study show that 75 mg [R]acetorphan tablet induces a plasmatic enkephalinase inhibition similar to [R,S]acetorphan 100 mg capsule.

- **Evolution of plasma enkephalinase activity after repeated administration of [R,S]acetorphan in man [6-7]**

Kinetic parameters after repeated administration have been evaluated according to two criteria :

- evolution of methylthioether of thiorphan concentration;
- evolution of enkephalinase activity.

([R,S]acetorphan 100 or 300 mg tid during 7 days)

In man as in animal, [R,S]acetorphan is rapidly metabolized in BP 0.35, active metabolite and then in methylthioether of BP 0.35, which is devoided of any pharmacological activity.
After 7 days of treatment (100 mg tid), the characteristics of the peak of the M metabolite are the following :

Table n° 1
Mean values of C_{max} and T_{max} of M metabolite at D7

	After [R,S]acetorphan 100 mg	
	1st administration	2nd administration
C_{max} (µg/ml) (m ± sd)	0.05 ± 0.01	0.12 ± 0.05
T_{max} (hr) (m ± sd)	2.5 ± 1.7	1.7 ± 0.5

These values are identical to those observed after the first administration of [R,S]acetorphan.

The cumulated 24 hour urinary excretion of the M metabolite is related to the dosage taken (100 or 300 mg) and does not differ between D1 and D7.

Table n° 2

Urinary elimination of M metabolite at D1 and D7

(m ± ESM)

	100 mg	300 mg
D1	21.9 ± 5.5	68.4 ± 9.4
D7	20.4 ± 4.8	62.7 ± 12.1

These results show that the urinary elimination of [R,S]acetorphan is not modified after a 7 day period treatment and that no accumulation occurs.

In the same study, the simultaneous measurement of enkephalinase activity shows the magnitude of the inhibition depending on the day of administration (D1 and D7) and the time of administration (first or third intake of the day). There is no tachyphylaxia or accumulation. The parallelism of the evolution of enkephalinase activity and M metabolite levels indicates that both could be used to follow the kinetic of [R,S]acetorphan.

- **Effect of food and Maalox on the pharmacokinetic of [R,S]acetorphan after a single oral administration of 100 mg in healthy volunteers [8]**

The ingestion of Maalox 30 minutes before the intake of [R,S]acetorphan (one capsule of 100 mg) does not modify the kinetic parameters of [R.S]acetorphan followed by a plasmatic enkephalinase activity. The ingestion of a meal just after the intake of [R,S]acetorphan delays the T_{max} for 90 minutes without any modification of the area under the curve.

- **Pharmacokinetic of [R,S]acetorphan in elderly [9]**

The pharmacokinetics of [R,S]acetorphan following the ingestion of 1 capsule (100 mg) does not differ between elderly and adults.

- **Effect of [R,S]acetorphan on Cytochrom P 450 activity [10]**

The pharmacokinetic parameters of the clearance of antipyrine are not modified before and after 8 days of treatment with [R,S]acetorphan (100 mg t.i.d.).

- **Pharmacokinetic of [R,S]acetorphan in association with loperamide or nifuroxazide [11]**

  The adjunction of loperamide or nifuroxazide does not modify the pharmacokinetic of [R,S]acetorphan.

In conclusion

- [R,S]acetorphan is well absorbed, distributed, metabolized and eliminated in urine. The kinetics profile is not modified by repeated administration or in elderly subjects. From these pharmacokinetic parameters, it would be concluded that a t.i.d. regimen of administration is the most suitable to achieve a sufficient enkephalinase inhibition predictable of a satisfactory clinical activity.

- [R]acetorphan 75 mg tablets induce a similar plasmatic enkephalinase inhibition than [R,S]acetorphan 100 mg capsule.

5.3 PHARMACOKINETICS IN CHILDREN

- **Pharmacokinetic study of [R,S]acetorphan in children aged from 1 month to 6 years old**

This open study was performed in six children. [R,S]acetorphan was given at a single dose (1% granulated powder, 1,5 mg/kg) just before food intake.

Blood samples were taken at T0, 1 hour, 2 hours, 4 hours, 8 hours after drug intake. The active metabolite, thiorphan, was dosed using specific radio-immuno assay.

Table n° 3

Plasmatic concentrations (individuel data) (nmol/l)

N°	T_0	T_1 = 1 hr	T_2 = 2 hrs	T_3 = 4 hrs	T_4 = 8 hrs
1	nd	176	150	75	41
2	nd	92	473	63	29
3	nd	29	128	17	6
4	nd	103	150	225	nd
5	nd	63	54	71	17
6	nd	nd	147	88	13
mean ± SEM	0	77 ± 25	184 ± 60	90 ± 29	18 ± 6

nd = not detectable

The absorption of [R,S]acetorphan is rapid and high concentrations of thiorphan, the active metabolite (≃ 80 nmol/l) are observed for at least 4 hours. Thiorphan is still detectable (18 nmol/l), 8 hours after the drug intake. Taking into account the thiorphan IC_{50} on enkephalinase (2 nM) and its protein binding (85 %), these calculated concentrations of free thiorphan are sufficient.

Table n° 4

Pharmacokinetic parameters after a single dose of [R,S]acetorphan

N	DOSE (mg/kg)	tmax (hour)	Cmax (nmol/l)	Cmin (nmol/l)	AUC 0-t (nmol.h/l)	Cmax norm (nmol/l)	AUC 0-t norm nmol.h/l)
Mean	1.37	2.50	203	18	583	229	651
± SEM	0.1	0.5	58	6	110	71	135
Median	1.29	2.00	162	15	532	170	571

norm : normalized value for theoretical dose of 1.5 mg/kg

Maximal concentrations and Area Under the Curve evidenced a moderate variability (20 %) between subject related to the relatively few patients enrolled.

The value of Tmax (2 h 30) is explained by the food intake as it has been shown in adult that the ingestion of a meal delayed the Tmax, 80 minutes (fed state) to 180 minutes (post prandial state). A meal does not significantly modify the Cmax and the AUC.

[1] HAZLETON U.K. (^{14}C)[R,S]Acétorphan : absorption, distribution, métabolisme et élimination après adminisration d'une dose unique par voie orale au rat pigmenté et non pigmenté. Dossier A.M.M., TIORFAN®, volume 19, rapport n° HUK 6028 708/2.

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]PELLO JY. Etude de l'effet du [R,S]Acétorphan par voie orale sur la clearance à l'antipyrine chez le volontaire sain. Dossier A.M.M. TIORFAN®, volume 38.

BERGMANN JF. Recherche d'une éventuelle interaction du lopéramide et du nifuroxazide sur la biodisponibilité et le profil pharmacocinétique du [R,S]Acétorphan par voie orale, en dose unique, chez le volontaire sain. "Données internes"

6. PHARMACODYNAMIC STUDIES

6.1 EFFECT OF [R,S]ACETORPHAN ON CASTOR OIL INDUCED DIARRHEA IN MAN (Double-blind versus placebo study) [1]

The aim of this study was to confirm in man the antidiarrheal activity of observed in animals.

Six healthy volunteers (three males, three females) absorbed either [R,S]acetorphan 12 mg/kg or placebo in each session at weekly intervals. Fourty five minutes after treatment, they absorbed 30 g of castor oil. The times of every stool was recorded during the following 24 hours and each stool was weighed.

Table n° 1 : Effects of [R,S]acetorphan on castor oil induced diarrhea

	Placebo	[R,S]acetorphan	Difference
Total stool weight (g/24 hrs)	672 (76)	426 (83)*	-[37 (9)] %
Number of stools (/24 hours)	3.7 (0.3)	1.8 (0.2)+	-[49 (6)] %
First stool delay (hr)	4.8 (1.1)	7.0 (0.9)NS	+[113 (59)] %

Values given as means (SEM)

* $P < 0.01$; + = 0.002 ; NS = not significant ($p = 0.142$)

There was a significant reduction of stool weight (- 37 %) under [R,S]acetorphan ($P = 0.009$), all 6 subjects considered individually having a lower stool weight during the [R,S]acetorphan session than during the placebo session. In addition, the mean number of stools during the trial was reduced by 50 % ($P < 0.002$) during the [R,S]acetorphan period as compared with the placebo session. There was also a tendancy towards an extended delay (+ 113 %) between administration of castor oil and passage of the first stool but the difference failed to reach significance.

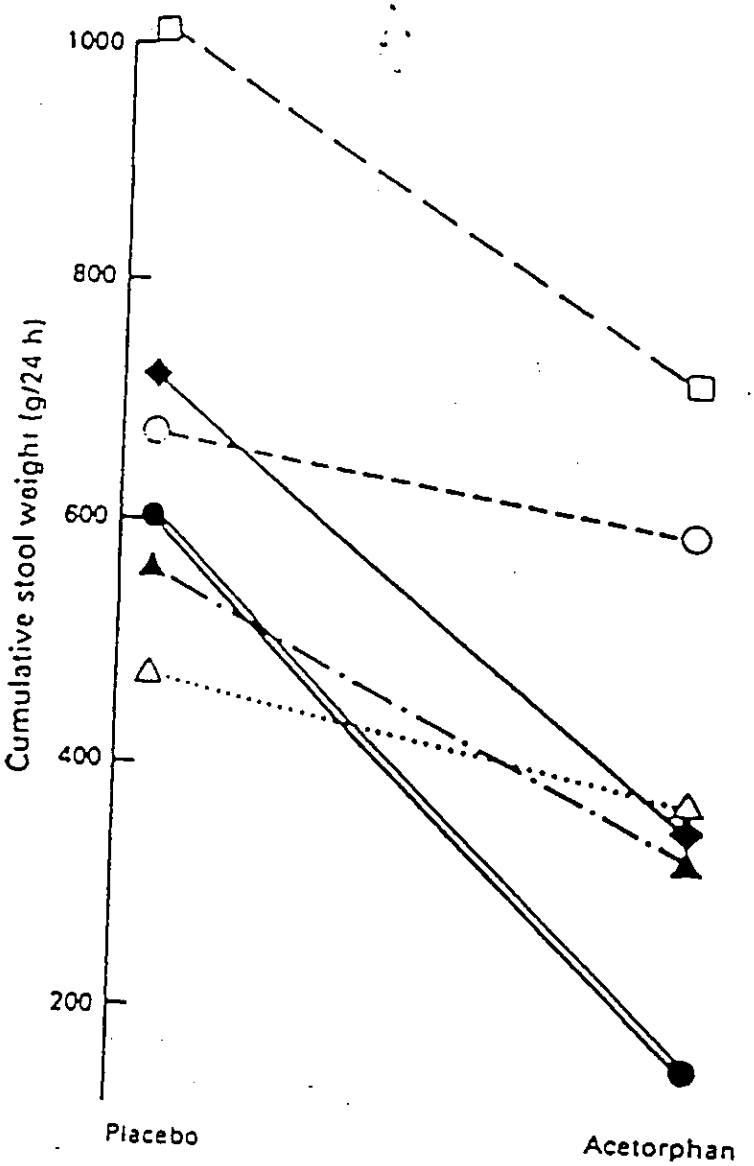


Fig 1 : Effect of [R,S]acetorphan on castor-oil induced diarrhea in six healthy volunteers : individual stool weights (g/24 h).

In conclusion, in human as in animals, [R,S]acetorphan prevents castor-oil induced diarrhea, a model of secretory diarrhea.

August 17, 1995

6.2 EFFECT OF [R,S]ACETORPHAN ON CHOLERA-TOXIN INDUCED DIARRHEA IN MAN [2]

The aim of this study was to confirm in man the intestinal antisecretory activity of [R,S]acetorphan demonstrated in animals.

Ten healthy volunteers (eight males, two females) were enrolled. Six received a single oral dose of [R,S]acetorphan 300 mg. Four served as control.

Hydro-electrolytic fluxes were measured on an isolated 30 cm jejunal segment

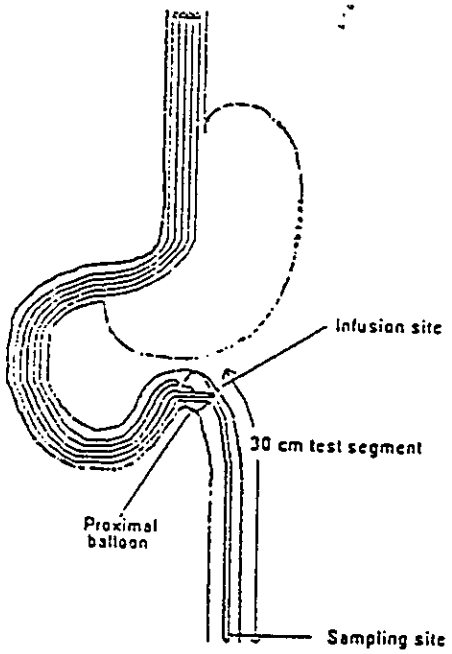


Figure 2 : Schema of the intestinal perfusion using a quadruple-lumen tube assemby with a proxim occluding balloon placed in the jejunum at the ligamentum of Tretiz

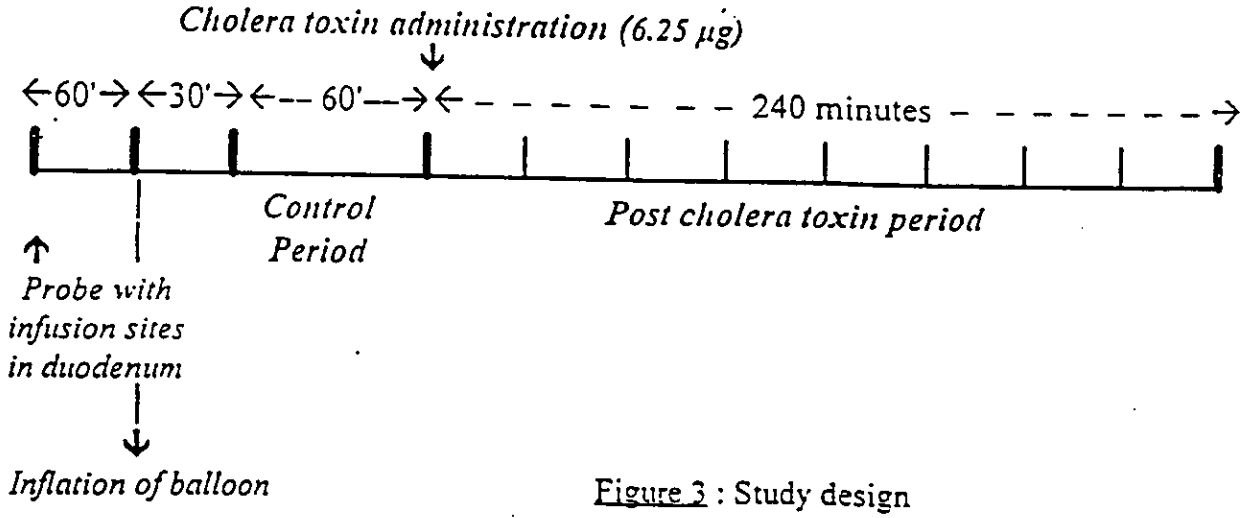


Figure 3 : Study design

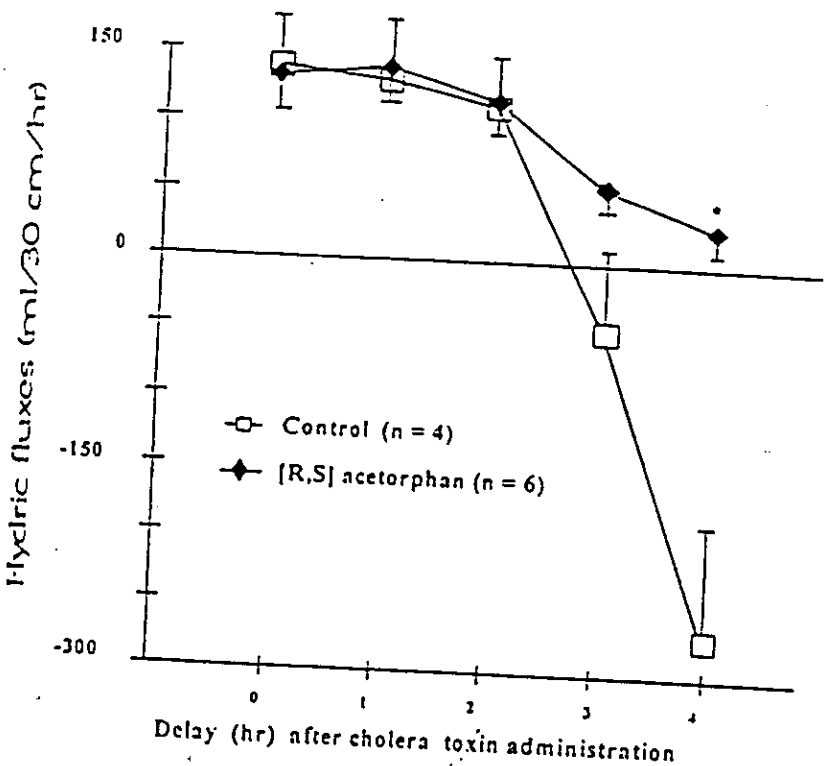
[R,S]acetorphan was orally taken 2 hours and 30 minutes before cholera toxin administration and electrolytic fluxes were measured during the following 4 hours.

Table 2

Electrolytic fluxes 4 hours after cholera toxin

	CONTROL	[R,S]ACETORPHAN
- Sodium (mmol)	- 43.5 ± 21.6	- 0.0 ± 2.0*
- Potassium (mmol)	- 0.9 ± 0.5	+ 0.1 + 0.1*
- Chlorides (mmol)	- 41.3 ± 21.3	- 4.3 ± 2.2*

* $P < 0.05$



$P < 0.05$

re 4 : Effect of [R,S]acetorphan on hydric fluxes after cholera toxin-induced diarrhea

After cholera toxin, [R,S]acetorphan abolishes cholera toxin-induced hypersecretion.

In conclusion, this study demonstrates in man the intestinal antisecretory activity of acetorphan as it has been previously demonstrated in animal.

6.3 EFFECT OF [R,S]ACETORPHAN ON OROCAECAL AND COLONIC TRANSIT TIMES [3]

The aim of this study was to confirm versus placebo in man the lack of effect of [R,S]acetorphan on oro-caecal and colonic transit times in healthy volunteers.

This double-blind cross over study was performed in 12 healthy males treated during 7 days with either [R,S]acetorphan 100 mg t.i.d. or placebo.

Colonic transit times were calculated by radiopaque markers method as described by Fotherby K.S. and Hunter J.O., 1987. Twenty radiopaque markers were swallowed every day during 5 consecutive days. On the sixth day, an abdominal X-ray was taken and radiopaque markers were retrieved.

Orocaecal transit time was measured on the seventh day by the appearance of sulfapyridine in plasma after oral administration of salicylazosulfapyridine according to J.E. Kellow, 1986.

Table 3

Orocaecal and colonic transit times in healthy volunteers after placebo and [R,S]acetorphan

		[R,S]ACETORPHAN	PLACEBO
TRANSIT TIMES :			
. OROCAECAL (min)		282 ± 27	303 ± 32
	TOTAL	31.3 ± 5.6	25.8 ± 5.8
. COLONIC (hrs)	Left colon	7.2 ± 1.4	5.0 ± 2.0
	Right colon	12.4 ± 4.0	9.8 ± 2.7
	Rectosigmoïd	11.7 ± 2.4	11.0 ± 3.6

means ± SEM

In conclusion, [R,S]acetorphan does not modify orocaecal and colonic transit times after multiple administration in healthy volunteers.

6.4 EFFECT OF [R,S]ACETORPHAN ON GASTRIC ACID SECRETION [4]

The effect of [R,S]acetorphan on gastric acid secretion was studied in a double-blind versus placebo study in 7 healthy volunteers.

Gastric acid secretion was stimulated with a protein-meal and with pentagastrin. [R,S]acetorphan was given in single dose by intraveinous route (3 mg/kg) and by oral route (15 mg/kg).

Table 4

Effect of [R,S]acetorphan on acid secretion and on integrated gastrinic response

	Placebo	[R,S]acetorphan i.v.	[R,S]acetorphan p.o.
Cumulative acid secretion (mmoles H^+/75 min)	29.5 ± 4.8	24.1 ± 3.8*	26.1 ± 4.0*
Integrated gastrinic response (pg/ml/75 min)	1 891 ± 147	2 161 ± 743	2 245 ± 1 085

* $P < 0.05$

[R,S]acetorphan significantly decreases acid secretion induced by a protein meal on a statistical point of vue but not on a clinical one. The lack of effect on integrated gastrinic response and on pentagastrin stimulated acid secretion suggests that [R,S]acetorphan acts on the non gastrinic pathway of the meal induced acid secretion.

EFFECT OF [R,S]ACETORPHAN ON GASTRIC EMPTYING

The lack of effect of [R,S]acetorphan on gastric emptying in healthy volunteers was demonstrated in 2 studies.

Effect of [R,S]acetorphan on gastric emptying of a liquid meal [5]

Compared to placebo, [R,S]acetorphan at a single dose (15 mg/kg/p.o. or 3 mg/kg/i.v.) does not modify the gastric emptying of a liquid meal using a double dilution technique.

. Effect of [R,S]acetorphan on gastric emptying of a solid meal [6]

After a single oral administration (10 mg/kg), [R,S]acetorphan does not modify gastric emptying of radiopaque pellets.

6.6 EFFECT OF [R,S]ACETORPHAN ON RECTAL AND ANAL MOTILITY

. Rectal electromyography [7]

A single intraveinous administration of [R,S]acetorphan (2 mg/kg) stimulates rectal electromyoelectrical activity in healthy volunteers.

. Anorectal motility [8]

A single oral administration of [R,S]acetorphan (5 to 30 mg/kg) does not modify anorectal manometric characteristics in healthy volunteers.

6.7 EFFECT OF [R,S]ACETORPHAN ON ESOPHAGEAL MOTILITY IN HEALTHY VOLUNTEERS [9]

An intraveinous infusion of [R,S]acetorphan (20 mg/kg for 20 minutes) compared to placebo in a double-blind study significantly decreases the lower esophageal sphincter relaxation (79.5 ± 2.9 %) versus (92.0 ± 2.6 %) ($P < 0.01$). The basal LOS pressure and the peristaltic waves were not significantly modified by [R,S]acetorphan.

6.8 COMPLEMENTARY PHARMACODYNAMIC STUDIES

Three studies confirm the specific affinity of [R,S]acetorphan for the digestive tract.

. Effect of [R,S]acetorphan on hormonal secretions in healthy volunteers [10]

A single oral dose of [R,S]acetorphan (100 mg) does not modify the plasmatic levels of serum thyreotrophin (TSH), growth hormone (GH), prolactine, luteinizing hormone (LH) and cortisol.

- Effect of [R,S]acetorphan on hypothalamo-pituito-adrenal axis in healthy volunteers [11]

In a double-blind study, the effect of [R,S]acetorphan (100 mg x 3/7 days) on basal and stimulated (metopyrone) function of the hypothalamus pituito-achenal axis was compared to the effect of a placebo. No modification of basal and stimulated secretion of cortisol, 11-desoxy-cortisol, ACTH, α-MSH, ß-LPH was observed under [R,S]acetorphan.

- Effect of [R,S]acetorphan on bronchoconstriction in asthmatic patients [12]

A single oral dose of [R,S]acetorphan (200 mg) given to asthmatic patients does not modify respiratory tests both in basal state and after bisulfite administration.

- Effect of [R,S]acetorphan on vigilance [13]

A double-blind versus placebo study was conducted in 12 healthy volunteers to look at the effect of [R,S]acetorphan on vigilance using psychometric tests and self-rating scales. The effect of [R,S]acetorphan was studied after a single administration (200 mg) and after repeated administration (3 days).

No effect of [R,S]acetorphan was noticed.

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7. CLINICAL STUDIES

STUDIES IN ACUTE DIARRHEA

OPEN MULTICENTRIC STUDY [1]

Ninety-two adult ambulatory patients with acute diarrhea [mean daily number of stools : 6.9 ± 0.3] lasting for less than 5 days were enrolled. The efficacy of treatment was evaluated on the delay of recovery, the cumulative probability of recovery, the number and characteristic of stools, the evolution of clinical symptoms. The results were the following :

- delay of recovery : 3.6 days,
- cumulative probability of recovery on day 5 : 85 %,
- mean number of abnormal clinical finding : 0.1 after treatment versus 1.5 at the inclusion time,
- therapeutic success in 90 % of cases.

DOUBLE-BLIND VERSUS PLACEBO STUDIES

Dose ranging study of [R,S]acetorphan (30, 100 and 300 mg t.i.d.) in acute diarrhea [2]

227 patients suffering from acute diarrhea (mean number of stools 6.0 ± 2.3) for less than 5 days were enrolled in this study. The aim of this study was to compare the efficacy and the safety of the three doses in comparison with a placebo.

The results showed a rapid efficacy of [R,S]acetorphan in comparison to placebo without any significant difference between the three doses tested.

Table 1 : Mean number of loose stools

THERAPEUTIC PERIOD	PLACEBO	[R,S]ACETORPHAN	P*
0 - 9 hours	2.7	2.1	0.06
0 - 60 hours	8.6	7.1	0.03

* ANOVA

30 mg was not different from the highest dosages, however diarrhea was less severe in this group as attested by a lower severity grading and work interruption.

Clinical and biological tolerance was good and does not differ between the 4 groups of treatment.

- French double-blind versus placebo study in acute diarrhea [3]

198 adult patients suffering from acute diarrhea (mean number of stools : 5) for less than five days were included in this double-blind versus placebo study.

The efficacy of treatment was evaluated on :

- the delay of recovery,
- the cumulative probability of recovery,
- the number and quality of stools,
- the clinical symptoms (abdominal pain, abdominal distension, nausea, anal burning) and clinical examination,
- an overall evaluation of efficacy and safety.

Results

[R,S]acetorphan was efficient on all the criteria of efficacy as it is shown in table and figure.

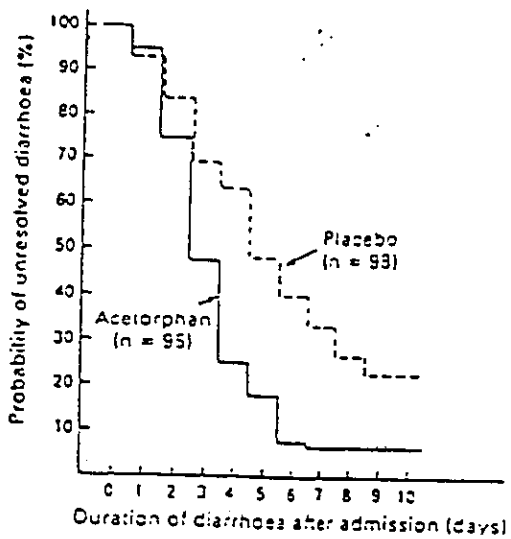

Figure 1 : Actuarial curves of diarrhea in 193 patients with acute diarrhea treated with [R,S]acetorphan or placebo. Comparison between the two treatment groups by log rank test, $p < 0.001$.

Table 2 : Effects of [R,S]acetorphan in acute diarrhea : symptoms and clinical signs at the end of the trial

SYMPTOMS AND SIGNS	[R,S]ACETORPHAN n (%)	PLACEBO n (%)
Diarrhea	7 (7.4)*	23 (23.5)+
Anal burning	3 (6.3)	11 (23.9)*
Spontaneous abdominal pain	8 (9.6)	18 (20.5)*
Nausea	5 (6.9)	11 (17;7)*
Anorexia	16 (20.3)	27 (35.5)*
Pain on abdominal distension	7 (8.5)	27 (32.5)@
Abdominal distension	13 (18.3)	26 (34.7)*
Fever	0 (0)	2 (5.4) NS

Data were recorded blindly during the second visit to the physician (10-14 days after inclusion). n = number of patients ; % = n x 100/number of patients who had this symptom or sign on visit 1. Comparison between [R,S]acetorphan and placebo groups using a two tailed Student's *t* test. * $P < 0.05$; + $P < 0.01$; @ $P < 0.001$; NS : Not Significant.

Tunisian double blind placebo controlled study [4]

The aim of this study was to demonstrate the efficacy of [R,S]acetorphan (100 mg, t.i.d.) in acute diarrhea on an objective criteria : stool weight.

70 patients suffering from acute diarrhea [mean number of stools : 6.4 ± 2.8] for less than 5 days, were enrolled in this double-blind versus placebo study.
The efficacy was evaluated on the daily number and characteristics of stool, on the stool weight during the first 24 hours under treatment. The clinical safety was evaluated by a clinical examination and the 24 hour stool weight collection after the recovery.

As it is shown in table 3 (next page), the rapid efficacy of [R,S]acetorphan is demonstrated by the significant decrease of the stool weight and of the number of stool during the first 24 hours of treatment.

Table 3 : Stool weight and number of stool during the first 24 hours of treatment

EVALUATION CRITERIA (m ± sd)	PLACEBO (n = 38)	[R,S]acetorphan (n = 32)	P
Stool number during the first 24 hours of treatment	4.7 ± 1.9	4.0 ± 1.7	< 0.05
Stool weight during the first 24 hours of treatment	506 ± 42	363 ± 54	< 0.05

After recovery, the stool weight does not differ between [R,S]acetorphan and placebo, it demonstrates the lack of secondary constipation under [R,S]acetorphan, it should be related to the lack of modification of intestinal transit time under [R,S]acetorphan.

DOUBLE BLIND VERSUS LOPERAMIDE STUDIES

Two double-blind versus placebo studies have been conducted to compare the efficacy and tolerance of [R,S]acetorphan and loperamide, a μ-receptor agonist in the treatment of acute diarrhea.

The aim of the first one [5], was mainly to compare the tolerance of the two compounds and especially the frequency of secondary constipation.

69 patients suffering from acute diarrhea of presumed infectious origin having started less than 5 days before, were enrolled in the study.

37 received [R,S]acetorphan, 4 capsules of 100 mg the first day and 3 capsules the following days until recovery.

32 received loperamide, 4 capsules of 1.33 mg the first day (5.3 mg) and 3 capsules the following days until recovery.

The main criterion of evolution was a safety one : the frequency of secondary constipation in the 2 groups of treatment.

The other criteria were :

- the delay of recovery,
- the overall evaluation of efficacy,
- the duration of associated symptoms mainly bloating and abdominal distension.

Results are expressed in the following table :

Table 4 : Comparison between the two treatments

	[R,S]acetorphan (n = 37)	LOPERAMIDE (n = 32)	P
Evaluation of efficacy by the physician (Excellent or good)	91.9 %	87.5 %	NS
Delay or recovery (day)	2.2 ± 0.2	2.3 ± 0.2	NS
Abdominal distension > 1 day	27 %	50 %	< 0.05
Abdominal pain > 1 day	40.5 %	59.4 %	= 0.1
Constipation after recovery of diarrhea	8.1 %	31.3 %	< 0.02

They showed that : the delay of recovery does not differ between the 2 treatments. Abdominal distension is significantly more rapidly improved by [R,S]acetorphan. There is also a tendency in favour of [R,S]acetorphan concerning the delay of recovery from abdominal pain. Concerning the tolerance, significantly less patients complained of constipation in the [R,S]acetorphan group as compared to the loperamide one.

In the second study, 157 patients suffering from acute diarrhea were enrolled.

This double-blind trial was conducted using a double dummy. The doses for [R,S]acetorphan, consisted in one 100 mg capsule initially, followed by 3 capsules daily, one before each main meal. For loperamide, two capsules (2 mg) initially and one capsule after each loose stool. Treatment was stopped after recovery.

The main criteria of evaluation were the severity and duration of diarrhea.

As shown in the figure n° 2 next page, the rapidity of diarrhea resolution was similar in the 2 groups of treatment.

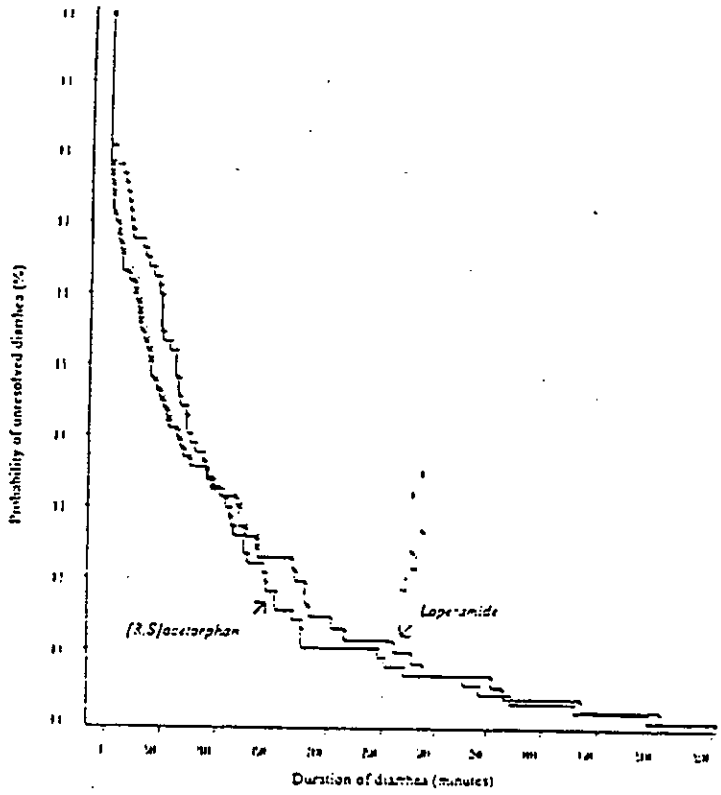

Figure 2 : Actuarial curves of diarrhea in 157 patients treated with [R,S]acetorphan or Loperamide

The 2 groups of treatment also did not differ for the other evaluation criteria.

Table 5: Characteristics of secondary constipation

	[R,S]acetorphan	Loperamide
% of constipated patients	39 % [n=32]	47 % [n=35]
Number of days without bowel movement (m ± SEM]	1.3 ± 0.1	1.6 ± 0.1
% of patients with at least 2 days without bowel movement	9.8 % [n=8]	18.7 % [n= 14]

These results showed :

- a similar efficacy for both treatment

- a tendency to a higher percentage of secondary constipation (defined as at least one day without bowel movement), particularly for prolonged one.

In conclusion, the different mechanisms of action of the 2 drugs have clinical consequences. The intestinal stasis under loperamide leads to : secondary constipation, bloating and abdominal pain. On the opposite, the pure intestinal antisecretory activity of acetorphan leads to a similar efficacy on diarrhea and to a quicker efficacy in abdominal symptoms.

7.2 STUDIES IN CHRONIC DIARRHEA

HIV+ DIARRHEA [6, 7]

Two studies were performed in HIV+ patients suffering from chronic diarrhea.

The first one was conducted in AIDS patients hospitalized for diarrhea. [R,S]acetorphan (100 to 300 mg, t.i.d.) and octreotide (50 to 150 µg, t.i.d.) were given in random order to 13 patients during 2 one-week periods. At inclusion, diarrhea lasted for 35 ± 8 weeks despite use of traditional antidiarrheal agents and was characterized by 7.0 ± 1.2 stools/day, weighing 1,033 ± 174 g/day, with lipid output of 18.8 ± 3.5 g/day. Response was defined as a reduction by at least one third of both daily stool number and weight.

The mean daily stool number was reduced to 4.6 ± 1.1 under [R,S]acetorphan ($P \leq 0.05$) but was 5.6 ± 1.2 under octreotide (NS). Whereas two patients responded to both treatments, two responded to [R,S]acetorphan alone and one to octreotide alone. In addition, gastrointestinal symptoms associated with diarrhea, e.g. nausea, anorexia and abdominal pain, were markedly reduced by both treatments. Daily lipid output in faeces, reflecting malabsorption, was reduced non significantly with [R,S]acetorphan (11.5 ± 2.3 g) but was nearly doubled with octreotide (33.7 ± 12.0 g). [R,S]acetorphan was very well tolerated.

[R,S]acetorphan significantly decreases the mean number of stools at the opposite of octreotide, the only reference treatment of severe AIDS diarrhea which is not effective in this study, furthermore, [R,S]acetorphan presents major advantages such as the oral route of administration and the diminution of lipid fecal loss in these cachectic patients.

The second one was conducted in 174 HIV+ patients with chronic diarrhea not making necessary an hospitalization. It was a double-blind placebo controlled study with parallel groups. Treatments were randomly allocated either placebo or acetorphan [100 to 200 mg t.i.d. per os]. Duration of treament was 2 to 4 weeks. No other antidiarrheal agent was allowed. Response was defined as a reduction by more than one third of stool number.

The 174 patients were HIV-infected for more than 4 years and their diarrhea (5 stools/day) lasted for 9 months. The per-protocol analysis gave the following results : the frequency of response was 36 % [26/72] with acetorphan and 23 % [17/74] with placebo (P = 0.08). On day 15, stool number was 4.0 ± 0.4 (n=64) with acetorphan and 5.0 ± 0.6 (n=68) with placebo (P = 0.07). On day 30, stool number was 2.4 ± 0.2 (n=18) with acetorphan and 3.7 ± 0.5 (n=23) with placebo (P = 0.02). Among patients without cryptosporidiosis, the only significant pronostic factor, the percentage of response was 41 % [24/58] with acetorphan and 24 % [13/55] with placebo(P = 0.045).

Acetorphan [300 to 600 mg/day during 2 to 4 weeks] is useful in the treatment of diarrhea in HIV-infected patients particularly in the absence of cryptosporidiosis.

CHEMOTHERAPY INDUCED DIARRHEA [8]

[R,S]acetorphan was studied on acute diarrhea chemotherapy-induced diarrhea in 12 patients treated with 5 FU and suffering from diarrhea (WHO grade 2 and 3). Patients received 3 capsules (100 mg) of [R,S]acetorphan.
Under treatment, the number of stools per day was reduced in all cases from 6.3 to 4.9 ($p < 0.002$) and the number of days with liquid stools dropped from 4.7 to 2.4 ($p < 0.02$).

In conclusion, these results suggest the efficacy of [R,S]acetorphan on chemotherapy-induced diarrhea.

7.3 STUDIES IN PEDIATRICS

RANDOMIZED, DOUBLE-BLIND, STUDY OF [R,S]ACETORPHAN IN COMPARISON WITH LOPERAMIDE FOR THE TREATMENT OF ACUTE DIARRHEA IN CHILDREN AGED FROM 2 TO 10

This study was a multicenter study in 102 children suffering from acute diarrhea, enrolled by 19 general practitioners.

The aim of this trial was to compare the efficacy and the safety of [R,S]acetorphan and loperamide in children older than 2 years, as loperamide is contra-indicated in children below 2 years.

The main efficacy criterion was the number of loose stools from the initiation of treatment to the recovery. Secondary criteria were : the duration of diarrhea, the number of concomitant treatments, the evolution of abdominal circumference and the frequency of occurrence of secondary constipation.

The posology and the galenical form were the usual ones for the 2 treatments, therefore a double-dummy was used : liquid form for loperamide and a granulated powder for [R,S]acetorphan. The [R,S]acetorphan was administered 1.5 mg t.i.d., loperamide 4 drops/kg t.i.d.

Fifety-two children received [R,S]acetorphan, and 50 received loperamide.

At inclusion, the 2 groups were not different, notably for the age :

Table n° 6

Repartition of children according to the age

AGE (YEARS)	[R,S]ACETORPHAN	LOPERAMIDE
2 - 4	27	24
4 - 6	9	13
6 - 12	16	13

ıere was also no difference for the duration of diarrhea before inclusion (2 days) and the ıber of diarrheic stools within the 24 hours before inclusion (5 stools).
e mean dose of [R,S]acetorphan was 1.34 ± 0.02 mg/kg per intake, t.i.d.
esults are expressed in the table next page.

Table n° 7
Efficacy of both treatments

	[R,S]ACETORPHAN	LOPERAMIDE	P
- Duration of diarrhea [hours]	10.7 ± 1.6	8.8 ± 2.2	NS
- Mean number of diarrheic stools for the duration of diarrhea	2.7 ± 0.4	2.1 ± 0.4	NS
- Duration of treatment [days]	1.9 ± 0.2	1.8 ± 0.2	NS
- Number of patients with a modification of associated treatments	10	19	= 0.04
- Number of constipated patients [24 hours without stools]	19	29	= 0.03

This study shows a similar efficacy for [R,S]acetorphan and loperamide, however, [R,S]acetorphan is significantly better than loperamide for 2 important criteria related to tolerance : the number of associated treatments which were needed, and the frequency of secondary constipation, both were significantly lower with [R,S]acetorphan. A lower frequency of secondary constipation was already observed in 2 studies conducted in adults in comparison with loperamide and is related to the lack of transit inhibition with acetorphan demonstrated both in animals and in man.

DOUBLE-BLIND PLACEBO-CONTROLLED STUDY OF ACETORPHAN IN CHILDREN AGED BETWEEN 3 MONTHS TO 4 YEARS OLD WITH ACUTE DIARRHEA.

This multicentre study included 172 children hospitalized for acute diarrhea in 13 centers in France.

The primary measure of criterion was stool output during the first 48 hours (stool weight / body weight). The secondary efficacy variables were stool output during the first 24 hours, duration of diarrhea, treatment duration and frequence of dehydration.

149 children were analysed for efficacy in whom 73 received acetorphan and 76 received placebo.

There was no difference between acetorphan group and placebo group in terms of age and body weight at inclusion. The mean age was 13 months with a minimum of 1.6 months and a maximum of 3.6 years old.

Table 8

	PLACEBO GROUP	ACETORPHAN GROUP
0 - 3 months	-	1
3 - 6 months	13	23
6 months - 1 year	34	32
1 year - 2 years	24	21
2 years - 4 years	12	12
MEAN AGE [month ± SEM]	13,6 ± 1,0	12,0 ± 0,9

There was no difference between acetorphan group and placebo group in terms of diarrhea duration (2 days) and number of stools during the last 24 hours before inclusion, which was 0 ± 0.3 stools in acetorphan group and 6.5 ± 0.4 stools in placebo group.

The bacteriological culture of the stools was performed in 162 children. A bacterial infection was found in 18% of children in acetorphan group and 12.5% of children in placebo group. A high prevalence of infections due to rotavirus was noted. The test was performed in a total of 136 children at inclusion and 67 of them had a positive result. This finding confirmed that the rotavirus was the common cause of diarrhea in young children during winter season.

The mean dose of acetorphan administered was 1.5 ± 0.03 mg/kg per time, 3 times per 24 hours.

The results are presented in following tables.

Table 9
Efficacy of acetorphan

CRITERIA	ACETORPHAN GROUP	PLACEBO GROUP	P
Stool output during the first 48 hours of treatment (g/kg) [mean ± SEM]	25 ± 4	76 ± 11	0.002
Stool output during the first 24 hours of treatment (g/kg) [mean ± SEM]	20 ± 3	45 ± 6	0.004
Frequency of dehydration [Na+/K+ < 1] after 24 hours (%) [mean ± SEM]	24.1	53.3	0.001
Duration of treatment (hours) [mean ± SEM]	40 ± 3	49 ± 3	0.02

Table 10
Efficacy of acetorphan in patients with rotavirus

CRITERIA	ACETORPHAN	PLACEBO	P
Stool output during the first 48 hours of treatment (g/kg) [mean ± SEM]	27 ± 7	102 ± 18	0.001
Stool output during the first 24 hours of treatment (g/kg) [mean ± SEM]	27 ± 7.4	58 ± 10	0.01
Duration of diarrhea (hours) [median]	6,9	36	0.02
Duration of treatment (hours) [mean ± SEM]	35 ± 4.3	49 ± 4,6	0.02

In conclusion, this study demonstrated that acetorphan reduced significantly stool output during the first 48 hours as well as 24 hours of treatment, duration of diarrhea, duration of treatment and frequency of dehydration in children with acute diarrhea.

OPEN STUDY OF PHARMACOKINETIC AND EFFICACY OF [R,S]ACETORPHAN

The aim of this study was to evaluate in parallel with the pharmacokinetic data also the efficacy of [R,S]acetorphan.

Ten children were enrolled in the study, they were hospitalized for severe diarrhea (7,5 liquid stools/24 hours at inclusion) lasting for more than 7 days.

[R,S]acetorphan has been administered at a mean dose of 1.45 ± 0,07 mg/kg and from one to six times.

The evolution of diarrhea (number and characteristics of stools) is described in the table below.

Table 11

DAILY NUMBER OF STOOLS AT INCLUSION		NUMBER OF STOOLS AT D1		NUMBER OF STOOLS AT D2	
Liquids	Overall	Liquids	Overall	Liquids	Overall
7.5 ± 0.6	7.5 ± 0.6	1.5 ± 0.5	3.1 ± 0.2	0	1.6 ± 0.2

(mean ± SEM)

This study confirms the efficacy of [R,S]acetorphan.

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8. TOLERANCE

8.1 CLINICAL TOLERANCE

[R,S]ACETORPHAN

PHASE I STUDIES

- Single dose up to 30 mg/kg, 20 fold the therapeutic dose, were administered and were perfectly well tolerated

- Repeated administrations of 15 mg/kg/day were also perfectly well tolerated.

PHASE II and IV STUDIES

2 364 subjects were treated with [R,S)acetorphan and among them, 493 were treated for at least 15 days, 430 for at least 1 month, 194 for at least 2 months and 100 for 3 months. As assessed in table 2 (page 3), the nature and the percentage of unexpected events do not differ between the [R,S)acetorphan and the placebo groups. The highest percentage of unexpected events concerns the digestive tract and are mainly related to the nature of the disease treated (Diarrhea or Irritable Bowel Syndrome).

POST MARKETING STUDY

1 500 000 patients have been treated.

Eight adverse effects have been reported in 7 patients :

Table 1

NATURE	FREQUENCY	IMPUTABILITY
Intestinal pseudo-obstruction	1/400 000	Plausible
Lip oedemia	1/400 000	Plausible
Facial oedemia	1/400 000	Plausible
Acute urinary retention	1/400 000	Plausible
Cutaneous eruption	2/400 000	Plausible
Cutaneous eruption	2/400 000	Uncertain
Hematuria	1/400 000	Uncertain

[R]ACETORPHAN

- 6 healthy volunteers received 50 mg three times
- 4 healthy volunteers 75 mg three times in single administration
- 9 healthy volunteers received 30 mg in single administration
- 25 patients received 300 mg/day during 6 weeks.

For these above studies, the tolerance was very good.

24 healthy volunteers received three doses (37.5, 75.0 and 150 mg) consecutively in single administration. Some minor and well tolerated adverse events were noted but not drug-related.

177 patients received 300 mg/day. It has not been noticed any serious adverse event or adverse event leading to study discontinuation. Only one adverse event related to the treatment (Abdominal pain and nausea quoted mild) has been reported.

12 patients received 300 mg/day during 7 days (Data analysis is in progress, at the time being, no serious adverse event has been noted).

Table 2 : Nature and frequency of unexpected events in all studies

NATURE	*PLACEBO* (n = 364)	*[R,S]ACETORPHAN* (n = 1 098)
igestive tract	49 (13.5 %)	96 (8.7 %)
. Nausea/Vomiting	19 (5.2 %)	34 (3.1 %)
. Epigastric pain	2 (0.5 %)	13 (1.2 %)
. Constipation	4 (1.1 %)	13 (1.2 %)
. Abdominal bloating	2 (0.5 %)	10 (0.9 %)
. Diarrhea	9 (2.5 %)	9 (0.8 %)
. Abdominal pain	6 (1.6 %)	8 (0.7 %)
. Miscellaneous	5 (1.4 %)	6 (0.5 %)
. Proctorrhagia	1 (0.3 %)	3 (0.3 %)
. Icterus	1 (0.3 %)	
ntral Nervous System	23 (6.3 %)	62 (5.6 %)
. Dizziness	7 (1.9 %)	15 (1.4 %)
. Asthenia	5 (1.4 %)	13 (1.2 %)
. Headaches	6 (1.6 %)	12 (1.0 %)
. Drowsiness		12 (1.0 %)
. Mood change	2 (0.5 %)	4 (0.4 %)
. Insomnia	1 (0.3 %)	3 (0.3 %)
. Faintness	1 (0.2 %)	2 (0.2 %)
. Nervous breakdown		1 (0.1 %)
. Convulsions	1 (0.3 %)	
n	7 (1.9 %)	10 (0.9 %)
. Skin rash	5 (1.4 %)	6 (0.5 %)
. Pruritus	1 (0.3 %)	1 (0.1 %)
. Acne	1 (0.3 %)	1 (0.1 %)
. Profuse sweating		1 (0.1 %)
. Sensitivity to sunlight		1 (0.1 %)
nephrology	2 (0.5 %)	6 (0.5 %)
Increase in the number of mictions		4 (0.4 %)
Decrease in the number of mictions		1 (0.1 %)
Cystitis	2 (0.5 %)	1 (0.1 %)
urrent infection	7 (1.9 %)	10 (0.9 %)
tion of food and water intake	9 (2.5 %)	9 (0.8 %)
laneous Stomatology	5 (1.4 %)	6 (0.5 %)
laneous Rheumatology	2 (0.5 %)	4 (0.4 %)
weight		3 (0.3 %)
llness		2 (0.2 %)
nsion	1 (0.3 %)	
e in intra-ocular e		1 (0.1 %)
	1 (0.3 %)	
	106 (29.1 %)	209 (19.0 %)

1995

[R,S]ACETORPHAN : PEDIATRICS FORMULATION

Clinical safety data were collected in 3 studies on 284 patients, among them 151 received [R,S]acetorphan.

Table 3

Repartition of the occurrence of side effects (except secondary constipation) according to the age

AGE	[R,S]ACETORPAHN	PLACEBO	LOPERAMIDE
- from 1 month to 2 years	7/86 (8.14 %)	9/71 (12.7 %)	--
including < 1 year	5/61 (8.19 %)	4/47 (8.51 %)	--
- from 2 to 12 years	8/65 (12.3 %)		11/50 (22 %)
TOTAL	15/151 (9.9 %)	9/83 (10.8 %)	11/50* (22 %)

* P = 0.03 versus [R,S]acetorphan

Table 4

Number and frequency of side events on the different groups of treatment

NATURE	PLACEBO	[R,S]ACETORPAHN	LOPERAMIDE
Vomitus	3	11	6
Fever	1	3	2
Abdominal pain	-	0	3
Cutaneous signs	4	0	2
Miscalleneous	3	3	2
TOTAL except Constipation	11/83 (13.25 %)	17/151 (11.26 %)	15/50* (30%)
Constipation	-	19/52 (36 %)	29/50* (58 %)

* P = 0.03 versus [R,S]acetorphan

No severe side event was observed under [R,S]acetorphan, the most frequent side event was vomitus, 11/151 (7.3 %) under [R,S]acetorphan, 9/133 (6.8 %) under placebo and/or loperamide.

The frequency of side-events is not different for [R,S]acetorphan (11.56 %) and for placebo (13.25 %), suggesting that they are related to acute diarrhea and not ot [R,S]acetorphan.

The frequency of side-events (except constipation) is significantly lower under [R,S]acetorphan (11.26 %) than loperamide (30 %), P = 0.03.

An episode of secondary constipation was observed more frequently under loperamide than under [R,S]acetorphan (P = 0,03), confirming the lack of transit inhibition under [R,S]acetorphan.

In children from 1 month to 2 years, the frequency of side-events under [R,S]acetorphan does not differ with placebo respectively 7/86 (8.14 %) and 9/71 (12.70 %) and shows the lack of Central Nervous System side event to the contrary of opiates which are contra-indicated below 2 years.

8.2 BIOLOGICAL TOLERANCE

The biological tolerance of [R,S]acetorphan was checked on hematology, biochemistry and hepatic tests performed in 52 healthy volunteers and in 496 patients and for a duration of treatment up to 90 days.

No significant individual abnormality of significant trend of biological parameters which could be related to [R,S]acetorphan have been observed.