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Attachment 1.

(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B. Date April 12, 1997

Principal Investigator H. Khan G Fuchs

Trainee Investigator (if any) _____

Application No. 97-010

Supporting Agency (if Non-ICDDR,B) _____

Title of Study A double-blind, randomized, placebo-controlled, dose response study to assess .... watery diarrhoea in adults.

Project status:
( ) New Study
( ) Continuation with change
( ) No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
   (a) Ill subjects (Yes) No
   (b) Non-ill subjects Yes No
   (c) Minors or persons under guardianship Yes No
2. Does the study involve:
   (a) Physical risks to the subjects (Yes) No
   (b) Social Risks Yes (No)
   (c) Psychological risks to subjects Yes (No)
   (d) Discomfort to subjects (Yes) No
   (e) Invasion of privacy Yes (No)
   (f) Disclosure of information damaging to subject or others Yes (No)
3. Does the study involve:
   (a) Use of records, (hospital, medical, death, birth or other) (Yes) No
   (b) Use of fetal tissue or abortus Yes (No)
   (c) Use of organs or body fluids (Yes) No
4. Are subjects clearly informed about:
   (a) Nature and purposes of study (Yes) No
   (b) Procedures to be followed including alternatives used (Yes) No
   (c) Physical risks (Yes) No
   (d) Sensitive questions (Yes) No
   (e) Benefits to be derived (Yes) No
   (f) Right to refuse to participate or to withdraw from study (Yes) No
   (g) Confidential handling of data (Yes) No
   (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure Yes No NA
5. Will signed consent form be required:
   (a) From subjects (Yes) No
   (b) From parent or guardian (if subjects are minors) Yes No
6. Will precautions be taken to protect anonymity of subjects (Yes) No
7. Check documents being submitted herewith to Committee:
   ___ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
   ☒ Protocol (Required)
   ☒ Abstract Summary (Required)
   ___ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
   ___ Informed consent form for subjects
   ___ Informed consent form for parent or guardian
   ___ Procedure for maintaining confidentiality
   ___ Questionnaire or interview schedule *

* If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
2. Examples of the type of specific questions to be asked in the sensitive areas.
3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

A Double-Blind, Randomized, Placebo-Controlled, Dose-Response Study To Assess The Efficacy Of Provir™ (SP-303) In The Treatment Of Acute Watery Diarrhea In Adults.

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USA

Cost

$154,239

PROTOCOL SUMMARY

Title:	**A Double-Blind, Randomized, Placebo-Controlled, Dose-Response Study To Assess The Efficacy Of SP-303 In The Treatment Of Acute Watery Diarrhea In Adults.**
Primary Objectives:	To assess the dose-response efficacy relationship of enteric-coated, compressed SP-303 tablets at daily doses of 0.5, 1.0, and 2.0 grams compared to a placebo capsule both administered orally in four divided doses for 48 hours.
Study Design:	A randomized, double-blind, placebo-controlled trial. Four groups of 35 patients per cohort will receive placebo or SP-303 (in daily doses of 0.5, 1.0, and 2.0 grams) enteric-coated tablets orally every 6 hours for 48 hours or a total of 8 doses.
Study Population:	Study participants will include a maximum of 140 evaluable subjects. The subjects will be otherwise healthy adult males or females between 18 and 70 years of age and who have resided uninterrupted in Bangladesh for $\geq$ 6 months. The subjects must be having on-going symptomatic acute diarrheal disease for less than 48 hours with $\geq$3 unformed (i.e., watery or soft) stools within the 24 hour period immediately prior to enrollment.
Study Procedures:	Subjects will be monitored for efficacy and tolerance parameters in the hospital for a duration of up to 96 hours (until resolution of diarrhea). Assessments will include clinical evaluation of including determination of stool frequency, stool volume and weight, stool consistency of every bowel movement, evaluation of gastrointestinal symptoms, and fluid requirements to maintain volume homeostasis after correction of dehydration at study entry and a record of adverse experiences.
Study Treatment:	Study subjects will be randomized according to severity of diarrheal disease (viz., associated with the degree of dehydration at the time of study enrollment) to receive orally a placebo capsule or a 0.5 gram SP-303, 1.0 gram SP-303, or 2.0 gram SP-303 given in equally divided doses every six hours for a total of 8 doses within 48 hrs.
Efficacy Evaluations	The primary efficacy outcome measurements will include a comparison between groups of total stool output, the time to last unformed stool, and the total number of unformed stools during the 48 hour treatment period. Secondary efficacy outcome measurements will include response between treatment and placebo groups in terms of stool frequency and consistency, need for unscheduled I.V., gastrointestinal symptoms, and fluid requirements to maintain volume homeostasis at 6 hour intervals over course of study. A significant difference is defined as $P<0.05$. Subjects will be evaluated on a daily basis during the surveillance period. The subjects may receive additional drugs or treatment for identified problems at the end of surveillance, if required.

I. BACKGROUND

Provir™ (SP-303) is an agent isolated from a plant of the family *Euphorbiaciae*. These species are widely distributed throughout Central and South America. The red, viscous latex from this plant is widely known for its medicinal properties (1-4). It has been used for many centuries by rural and urban populations in Central and South America. Its most common usage includes oral administration for relief of coughs, flu, "problems with lungs", diarrhea and "stomach ulcers". In fact, it has been reported that administration of 20 drops of the crude latex from *Croton lechileri* is associated with symptomatic relief of acute diarrhea. When estimating the amount of SP-303 that would be contained in this volume of latex from this plant based on butanol extraction (Shaman Pharmaceuticals, Inc., unpublished data), it would not exceed a few hundred mg of SP-303 per day. It should, however, be noted that the dose-response relationship of crude latex from this plant to effect symptomatic relief of diarrhea has not been rigorously assessed. No side-effects have been reported after routine internal use of the crude latex from the, according to ethnobotanists who have interviewed indigenous populations using this drug (4). Also, they report that there have been no serious, life-threatening complications associated with its usage. It has also been used in topical applications, as a healing agent for cuts, open sores, for the gums after tooth extraction, for oral open sores in and on the mouth and herpes infections.

SP-303 is a large proanthocyanidin oligomer which has been isolated from the latex of the plant species *Croton lechileri* (*Euphorbiacerae*) by scientists from Shaman Pharmaceuticals, Inc., in 1994 (5). In brief, a biologically active form is extracted from the latex by butanol and is subsequently purified by a series of chromatographic techniques. Chemical analyses confirm that SP-303 is a heterogeneous phenolic polymer. The polymer chains consist of linear chains of 5,7,3',4'-tetrahydroxy (catechin or epicatechin) or 5,6,3',4',5'-pentahydroxy flavan-3-ol (gallocatechin or galloepicatechin) units linked together through either C-4 to C-6 and/or C-4 to C-8. Catechin and gallocatechin chains predominate in SP-303 preparations. The sequence of the individual monomers within each chain have not been determined to date. Also, the visible spectral data strongly indicate the presence of a flavylium (anthocyanidin) moiety, although the relatively small fraction (<1% v/m) of this component has been an impediment in determining its position within the oligomer. The average chain length of SP-303 has been determined as being a heptamer by ^{13}C-nuclear magnetic resonance (NMR) via integration of the terminal unit/internal units. Negative ion fast atom bombardment mass spectrum (FABMS) has also allowed estimation of the relative proportions of oxidation within each of the chain lengths, as well as their average size range (up to 11-mer). The sum of these data supports an average molecular weight of 2,100 Da. The relative stereochemistry of SP-303 ranges from 2:1 to 1:2 ratios of catechin to epicatechin substructures, with the terminal unit having the epicatechin structure. The absolute stereochemistry of SP-303 determined from circular dichroism (CD) is consistent with the majority of other naturally occurring flavanoids at C-2. In addition, CD data confirm the chiral nature of SP-303. Biologically active SP-303 is acid-labile .

A. Preclinical Toxicity

The toxicity of SP-303 has been studied in several animal species following intravenous, intraperitoneal, oral and topical administration. The drug was well tolerated at oral doses as high as 500 mg/kg/day for 14 days and 200 mg/kg/day for 30 days in rodents and dogs, respectively. Orally administered to Rhesus monkeys the No-Observed-Effect-Level (NOEL) was between 100 and 200 mg/kg/day. Among the Rhesus monkeys, oral administration of 100 and 200 mg/kg/day for 30 days was associated with increased

occurrence of loose or liquid stools, pigmentation of hepatic sinusoidal cells, and greenish pigmentation of the histiocytes at all levels of the intestinal tract (from stomach to the rectum). All of these symptoms or findings which are of uncertain clinical significance subsided or disappeared by the end of the 30-day recovery period.

B. Mechanism of Action

Under the normal influence of the intracellular messengers cAMP and cGMP and ionized calcium, a protein channel in the apical membrane of the enterocyte opens and allows the egress of chloride ion (Cl^-) from inside the cell into the intestinal lumen down a concentration gradient. This chloride channel is also termed the cystic fibrosis transmembrane regulator. This is an electrogenic mechanism, with sodium (Na^+), potassium (K^+), and water following passively through tight junctions. The translocation of Cl^- into the gastrointestinal lumen is the driving force for the intestinal secretion of water. Classical infectious causes for the secretory watery diarrhea syndrome include the action of bacterial toxins (viz., holotoxin of *Vibrio cholerae* 01 and 0139 strains and heat-labile and/or heat-stable enterotoxins of *Escherichia coli* strains) which increase levels of cyclic nucleotides that both stimulate chloride secretion and inhibit neutral sodium chloride absorption (7). The other well-recognized mechanism of watery diarrhea syndrome due to an infectious agent is malabsorption of carbohydrates, primarily in the small bowel (8). This most commonly occurs in diarrhea caused by rotavirus among children. Whether chloride ion secretion has also a role in the watery diarrhea syndrome of rotavirus diarrhea is not known; however, stool chloride concentrations are significantly elevated in the stool of such children compared to normal stool (see Table 1). Furthermore, the role of chloride secretion in the watery diarrhea syndrome of other nontoxigenic infections (8-10), including AIDS-associated diarrhea, is not known.

It is well recognized that a pharmacological agent capable of inhibiting secretion of chloride in response to bacterial enterotoxins, as well as maybe many other infectious diarrheas, would be a potentially valuable adjunct to conventional rehydration therapy in patients with secretory watery diarrhea syndrome. The fact that SP-303 has recently been shown to be a specific chloride channel blocker has generated the impetus to evaluate its efficacy in the treatment of the watery diarrhea syndrome. The data to suggest that SP-303 inhibits gastrointestinal epithelial chloride secretion have been obtained by electrophysiological studies and studies in animal models of toxin-mediated secretory diarrhea: SP-303 has been demonstrated to reduce basal and stimulated transmembrane chloride-dependent current flux across intestinal tissue- culture Caco-2 and T84 cells under *in vitro* conditions utilizing an Ussing chamber (Dr. Sharif Gabriel, University of North Carolina, unpublished data; Dr. Edward Rozhon, Shaman Pharmaceuticals, unpublished data). It has been shown that SP-303 reverses the current stimulated by forskolin, a known stimulator of c-AMP-mediated chloride ion secretion, using this *in vitro* condition. This phenomenon was not irreversible since the chloride current of SP-303-stimulated cells can be reversed by the administration of forskolin. Furthermore, single channel electrophysiology experiments suggest that SP-303 blocks the cystic fibrosis transmembrane regulator (chloride ion channel) when in an open position (Gabriel, unpublished data; Dr. Phyllis Gardener, Stanford University, unpublished data). This inhibition is sufficient to block chloride flux from epithelial cells and the epithelial plasma membrane. However, SP-303 did not irreversibly close affected chloride channels since 15 minutes after washing-out SP-303 was sufficient to allow for identification of operative channels. In addition, orally administered SP-303 reduces cholera toxin-induced intestinal fluid accumulation in a sealed mouse model 3 hours after cholera toxin had already been administered (Dr. Sharif Gabriel, unpublished data; Dr. Edward Rozhon, Shaman

Pharmaceuticals, Inc., unpublished data). The timing of SP-303 administration in this animal study corresponds to the period when maximal fluid accumulation is occurring after cholera toxin stimulation. Similar results of decreased fluid accumulation into the gastrointestinal tract after a 6 to 8 hour experimental duration were obtained when suckling mice were administered SP-303 between 3 and 4 hours after exposure to heat-stable enterotoxin of *Escherichia coli* (Dr. Gary Schoolnik, Stanford University, unpublished data). These data collectively support the hypothesis that SP-303 can selectively inhibit gastrointestinal chloride secretion in unstimulated and stimulated cells.

C. Pharmacological Effects

The ancillary pharmacological effect of SP-303 has been evaluated in a series of *in vitro* and *in vivo* experiments designed to characterize its pharmacological profile. In a basic screen of radioligand receptor binding assays, SP-303 did not demonstrate any significant binding to adenosine, adrenergic, biogenic amine, amino acid, cholinergic, opiate, prostanoid, or second messenger receptors. In a series of basic *in vivo* screening experiments, SP-303 was devoid of significant inotropic, antiarrhythmic, anticonvulsant, hematologic, anti-gastrointestinal motility, or anti-inflammatory effects. SP-303 did not reduce gastric acid production or serum cholesterol. Intravenously, SP-303 was shown to produce a slight reduction (-9.4%) in heart rate at a dose of 30 mg/kg in rats, but there were no associated effects on systolic blood pressure. An intravenous dose of 10 mg/kg in mice induced a reduction in motor activity and an increase in grooming and stereotypic behavior. SP-303 produced a slight analgesic effect following oral and intravenous administration to rats using hot-plate and tail-flick assays, but had little effect in an acetylcholine writhing assay. SP-303 has been shown to possess antiviral activity against a broad range of respiratory viruses, including respiratory syncytial virus (RSV), influenza A and B, rotavirus, Herpes simplex, and parainfluenza types 1 andÊ3. The molecular basis for antiviral effect is not known. Despite the antiviral effects of SP-303, it had no inhibitory effects against a limited number of fungi (qs @ 1,000Ê_g/ml) or a limited number of bacterial species (qs @ 30 _g/ml).

D. Clinical Studies

SP-303 has been orally administered to more than 91 human volunteers in a total of seven Phase I studies involving adult volunteers, one Phase II study involving 30 pediatric patients, and one open-label Phase II study involving 75 patients with either traveler's diarrhea among adult U.S. citizens traveling to Mexico or non-specific diarrhea among adult Mexican nationals who permanently reside in Mexico. All clinical studies evaluating SP-303 in normal subjects or patients with respiratory synctial viral infection or diarrhea were in full-compliance with the regulations of the United States Food and Drug Administration to pursue clinical research under an IND application.

The SP-303 doses tested in the initial six Phase I studies ranged from 0.3 to 20 mg/kg as a single oral dose up to 27 mg/kg/day for seven days in a multiple oral dose protocol. An additional Phase I study was recently completed in healthy adults. This study evaluated safety after a single 1,250 mg SP-303 oral dose. The drug was well tolerated, including oral administration of 1,200 or 2,000 mg once daily for 7 days. There were minor adverse experiences confined to the digestive tract (viz., vague gastrointestinal complaints and nausea which was dose dependent). SP-303 has been administered as an aqueous solution (with or without 0.2% Xanthan gum to improve palatability) in six Phase I studies in healthy adult male volunteers and one prior Phase II study in children with lower respiratory tract infection. In the last completed Phase I and Phase II studies, the drug was administered in an enteric-coated bead, capsulated formulation.

In the first Phase I study, sequential groups of three or four adult male volunteers received single oral doses of 0.3, 1.0, 3.0 or 10.0 mg/kg SP-303 (as an aqueous solution) in an open label fashion. Asymptomatic elevations of hepatic transaminase levels (i.e., SGOT and SGPT) were observed at all dose levels. The elevations in hepatic transaminase levels were dose dependent and were more marked in the SGPT fraction. There were no other clinically remarkable changes in serum chemistry. Also, there was no evidence of significant abnormalities in vital signs, electrocardiograms, the hematological profile, urine samples, or adverse reactions.

The second study was a single-blind trial, conducted to further define the maximum tolerated dosage with respect to producing elevations in hepatic transaminase fractions. In the first treatment group, 12 subjects were randomly assigned to receive either a single 3 mg/kg SP-303 dose (n = 8) or a control solution (n = 4). Subsequent treatment groups received doses of 10, 15, and 20 mg/kg. The results from this trial did not confirm the results observed in the first trial with respect to elevations in hepatic transaminases. In this trial, the dose-related effects appeared to be nausea and vomiting at single oral doses of 15 to 20 mg/kg.

Twenty-four young healthy adult volunteers participated in the third Phase I trial, a rising multiple-dose tolerance evaluation of SP-303 administered at doses of 150, 300, and 600 mg once daily for seven days. No clinically significant or dose-related adverse experiences were reported. Administration of SP-303 once daily for 7 days at these doses did not produce any laboratory evidence of drug-related toxicity.

Sixteen young healthy adult male volunteers participated in the fourth Phase I study, a rising multiple dose tolerance study. Eight subjects participated in each of two sequential treatment groups in which six subjects received SP-303 and two subjects received the control solution. SP-303 was well tolerated following administration of 1,200 or 2,000 mg once daily for seven days. Adverse experiences of the digestive and nervous system were reported more frequently by subjects receiving SP-303 than by subjects in the control group. The occurrence of nausea was dose-related. All adverse experiences were self-limited and were mild or moderate in severity; none resulted in discontinuation of the study drug.

Thirty-two young healthy volunteers participated in the fifth Phase I study, a randomized, double-blind evaluation of the effect of oral SP-303 on viral shedding and clinical symptoms following experimental RSV infection. Eleven subjects received 75 mg SP-303 every 12 hours, 11 received 375 mg SP-303 every 12 hours, and 10 received the control solution every 12 hours. All subjects were treated for 7 consecutive days, beginning 2 to 3 hours before inoculation with low-passage, wild-type human respiratory syncytial virus. The effect of the drug on the incidence and level of virus shedding, or on the clinical signs and symptoms of respiratory illness could not be adequately determined due to the low incidence of viral shedding and signs of respiratory illness. SP-303 was well tolerated at doses of 75 and 375 mg every 12 hours for 7 days. With the exception of vomiting, which occurred only in the high dosage group, no apparent drug- or dose-related effects were observed.

The sixth study was a randomized, multi-center study to evaluate the pharmacokinetics and tolerance of escalating doses of Provir™ (SP-303) in infants and children with acute lower respiratory infection due to RSV. A total of 30 male or female children (age 3 to 24 months) were enrolled in the study and received doses ranging from 1.0 to 20.0 mg/kg/day for up to three days (in two equally divided doses). There were no reports of serious adverse events and most reported experiences were mild or moderate. Only one adverse experience was considered possibly related to the study medication, a single instance of

vomiting in one patient at the 2 mg/kg/day dose. These data indicate that SP-303 is safe when administered to young children with acute lower respiratory infection in doses up to 20 mg/kg per day for periods up to three days. No clinically significant levels of SP-303 were detected in the plasma or urine of the patients.

An additional Phase I study to determine the concentration of SP-303 in plasma and urine under more controlled analytical conditions was conducted in five healthy human male volunteers. Each subject received 1,000 mg SP-303 as an aqueous solution in 0.2% Xanthan gum, every 12 hours for seven days. SP-303 was well tolerated and no adverse experiences were reported. No SP-303 could be detected in the plasma or urine of these subjects.

Data from these studies have indicated that SP-303 is not absorbed in any appreciably amounts following oral administration. Due to the lack of palatability of aqueous solutions of SP-303, the drug was formulated in gelatin capsules and tablets for oral administration. In addition, due to the acid labile nature of SP-303, these formulations have been enteric-coated. To date, only the enteric-coated SP-303 beads have been administered to humans.

A recently completed Phase I study was conducted in six healthy adult male volunteers. They were administered a single, oral dose of gelatin capsules containing a total of 1,250 mg SP-303 in an enteric-coated bead formulation. The study was conducted to determine the concentration of SP-303 in plasma samples. Utilizing a sensitive high pressure liquid chromatography assay, SP-303 was not detected in plasma at levels above the lower limits of quantization. Also, none of these six volunteers experienced any serious adverse events. Furthermore, among the 75 patients with acute watery diarrhea syndrome who received SP-303 in an identical formulation as this seventh Phase I study, there were no serious drug-related adverse reactions after administration of a 1,250 mg loading dose followed by multiple doses that totaled 1 to 2 grams daily for a duration of two days. Based on these results of the last 81 patients, administration of the enteric-coated SP-303 bead formulation is well-tolerated after oral administration of single doses of 1,250 mg and multiple doses that total 1 to 2 gm per day for a total of two days duration.

E. Rationale for the Study

The justification for this study is based on the promising results obtained by Dr. Herbert DuPont from the Baylor College of Medicine, Houston, TX and the University of Texas Health Science Center, Houston, TX. These results were obtained in a clinical trial entitled: An Open-Label Pilot Study to Investigate the Safety and Effectiveness of Orally Administered Provir™ Capsules in the Symptomatic Treatment of Acute Nonspecific Diarrhea and Traveler's Diarrhea. The trial was initiated in May 1996 and completed by October 1996. A total of 75 patients with acute watery diarrhea were enrolled into this trial: 25 travelers from the U.S. to Mexico and 50 adult Mexican nationals who permanently reside in Mexico. They received SP-303 orally, 1 gm or 2 gm per day for a total of 2 days. In 24 of the 25 U.S. travelers and 19 of 50 permanent adult reidents of Mexico, an oral loading dose of 1,250 mg SP-303 was administered, followed every 6 hours by oral doses of SP-303 250 mg during the first 24 hours of treatment. Thereafter, these patients received SP-303 500 mg every 6 hours during the second day of treatment. Among the remaining volunteers, 16 adult Mexican nationals received 500 mg SP-303 every 6 hours for a total of 8 doses; and one traveler and 15 adult Mexican nationals received 250 mg SP-303 every 6 hours for a total of 8 doses. The SP-303 formulation used in this trial was gelatin capsules filled with enteric-coated SP-303 beads. Patients

were monitored for safety and efficacy (i.e., in terms of stool frequency, stool consistency, gastrointestinal symptoms) for a total of 3 days from the start of study drug administration.

In brief, SP-303 treatment utilizing an enteric-coated SP-303 bead formulation and SP-303 daily doses of 2 to 1 gm appear to be associated with a high rate of overall effectiveness (~90%) in this trial. SP-303 appears to reduce disease severity, disease duration, and disease symptoms by the end of 48 hours of treatment in both target populations. Seventy-two percent of the travelers returned to normal stool frequency by 48 hours after the start of treatment; >80% of the Mexican nationals returned to normal stool frequency even by 24 hours after the start of treatment. For comparison, based on the results of placebo-controlled historical clinical trials, it takes usually up to 96 hours and 48 hours for U.S. traveler's with diarrhea and Mexican nationals with diarrhea, respectively, to return to normal stool frequency (Dr. DuPont, unpublished data). SP-303 treatment in the two target populations suggests a clinically efficacious response. For example, it took only 30 hours for the time to last unformed stool to occur among SP-303 treated U.S. travelers to Mexico compared to a historical mean of 69 hours for placebo-controlled patients in three-day surveillance clinical trials. Similarly, the time to last unformed stool in Mexican nationals with watery diarrhea treated with either SP-303 1 gm daily or SP-303 2 gm daily was <24 hours compared to a historical mean of 32 to 38 hours for placebo-controlled patients in two- or three-day surveillance clinical trials, respectively (DuPont, unpublished data). Also, almost all treated patients with SP-303 were symptomatically improved after one day of SP-303 treatment. None of the SP-303 treated patients had any serious adverse reactions, sufficient to discontinue therapy or warrant intervention. In addition, there were no clinically significant abnormalities noted in hematological, serum chemistry, or urinalysis profiles three days after the start of the study compared to baseline levels at the time of study entry. It would appear, therefore, that oral SP-303 treatment in doses of 1 to 2 gm daily for two days is safe and well-tolerated in four divided doses or with a loading dose, as well as possibly associated with efficacy against watery diarrhea syndrome in indigenous and expatriate populations.

An over-riding impetus to purse these studies is related to the magnitude of the diarrheal disease problem, both in terms morbidity and mortality. It is estimated that 4 million childhood deaths occur per annum due to diarrheal diseases (11). Death is attributed commonly to the consequences of acute hypovolemia (i.e., acute fluid loss that is >10% of total body weight) and severe electrolyte abnormalities. In the United States and other developed countries, acute infectious diarrheal diseases are also a major cause of morbidity among children and the elderly, often resulting in hospitalization. It is estimated that acute diarrheal disease is responsible for >250,000 hospital admissions annually in the U.S., especially among children and the elderly (12). Furthermore, it is estimated that diarrheal illness is responsible for >8 million office visits to physicians by afflicted individuals of all ages each year in the United States. Diarrheal disease accounts for the third most common illness in the United States for which medical advice or care is sought or provided, respectively. It is, therefore, obvious that infectious diarrheal disease is an important international health problem in terms of mortality, morbidity, and public health.

The severity of this disease varies widely, irrespective of the etiologic agent, although it is easily recognized clinically. Diarrhea is described frequently as an abnormal increase in stool liquidity and frequency. For example, patients with *cholera gravis* can produce >25 liters of rice-water stool and more than 30 bowel movements per day. Fluid loss from the bowel involves the outpouring of watery fluids from the small bowel that are rich in electrolytes (see Table 1). Despite the dramatic fluid loss and stool frequency observed in certain infectious causes of diarrhea, diarrhea is formally defined in adults as an increase in daily stool weight above 200 gm. Despite the differences in daily stool weight because of differences in gender, diet, activity, or stress, the formal definition of diarrhea

provides a clear objective standard to establish normal versus abnormal stool volume (13).

Patients with acute infectious diarrhea typically present with nausea, vomiting, abdominal pain, fever, and diarrhea--- which may be watery, malabsorptive, or bloody, depending on the cause. Overall, this clinical entity is self-limited with an average of 4 to 7 days of acute illness followed by a rapid convalescent phase to full-recovery by 7 to 10 days in otherwise healthy individuals.

Patients with toxigenic infection (e.g., due to *V. cholerae* 01 or heat-labile and/or heat-stable producing enterotoxigenic *E. coli* strains [14]) typically have nausea and vomiting as prominent symptoms but rarely a high fever. The epidemiology of cholera and enterotoxigenic *E. coli* is well-known. The disease is usually localized in areas of poor sanitation and hygiene. In developing countries, these infections constitute a major proportion of the diarrheal illness in the population. For example, ~40% of all acute diarrheal disease in patients less than 25 years of age seeking medical care in Ujung Pandang, South Sulawesi (Indonesia) and Jakarta, the capital city on Java (Indonesia) are due to *V. cholerae* 01 strains or enterotoxigenic *E. coli* strains (O'Hanley, unpublished data). The proportion of acute diarrheal disease due to these enteric pathogens is reduced in older age groups (i.e., 26-40 years) as a consequence of acquired natural immunity; however, they still constitute a significant proportion of the etiologic cause for diarrhea in this age group who seek medical care (e.g., ~15%) or hospitalization (e.g., ~80%) (O'Hanley, unpublished data). These enteric pathogens constitute the classical cause of a watery, secretory diarrhea syndrome (14). The molecular basis for this syndrome is the secretion of chloride into the intestinal lumen with the passive efflux of water into the lumen leading to watery diarrhea. This type of diarrheal disease is self-limited (i.e., resolves in 5 to 7 days due to normal immune mechanisms). Therefore, the primary treatment objective is designed to establish or preserve homeostasis prior to the contributions of elicited immunity. It should be mentioned that approximately 50-60% of the adult individuals who seek medical care for acute watery diarrhea in Indonesia do not have an identified pathogen.

In contrast, most invasive enteropathogens (e.g., *Campylobacter*, *Salmonella*, and *Shigella*) and organisms that produce cytotoxins like *Clostridium difficile* or enterohemorrhagic *E. coli* strains cause fever, as well as intestinal inflammation and abdominal pain (15). The major exception to this rule is the diarrheal syndrome caused by enteroinvasive *E. coli*. In this syndrome, the patient does not usually manifest a fever despite invasive, inflammatory diarrhea. The treatment goal for all inflammatory/invasive type of diarrheal syndrome is to maintain homeostasis. However, there is a more urgent need to remove the infectious agent from the intestinal tract through antimicrobial administration since greater morbidity is likely to occur prior to the development of sufficient immune mechanisms of the host to clear the microorganism from the intestinal tract or neutralize the noxious effects of cytotoxins (16). Whether there is a role for chloride secretion into the intestinal lumen in the inflammatory diarrheal syndrome is not known; however, the possibility exists that chloride channels might be stimulated by microbial factors of invasive enteropathogens or other microbial products when the intestinal mucosa is injured. Furthermore, the role for chloride secretion in parasitic infections (e.g., *Cryptosporidium*, *Microsporidium*, *Isospora belli*, *Entamoeba histolytica*, *Giardia lamblia*, and *Cyclospora*) is not known but should be explored in view of the watery nature of the diarrheal syndrome in these infections.

The keystone to treating diarrhea, irrespective of the etiologic agent, is to establish and maintain homeostasis (17). For patients seeking medical care for diarrheal disease, clinical assessment must be done initially to determine the degree of dehydration. This

allows the clinician to decide whether intravenous or oral rehydration fluids are indicated. Patients who demonstrate severe dehydration (i.e., rapid pulse, decreased blood pressure, poor skin turgor, sunken eyes, muscle cramps, and decreased alterness) often require rapid intravenous fluid replacement (18). Such patients have a minimum fluid deficit equivalent to 10% of body weight. All others with lesser degrees of dehydration (i.e., rapid pulse but normal blood pressure, slightly decreased skin turgor, dry mouth) or no detectable dehydration can be treated with oral fluid replacement (18).

Rapid correction of severe dehydration and maintenance of hydration among patients with diarrhea can be accomplished by intravenous fluid. For initial therapy, Ringer's lactate or a similar preparation such as the Dhaka Solution is useful in all age groups (19). It is never acceptable to administer intravenous 5% glucose in water and any fluids with less than 90mEq/L of sodium. Thereafter, replacement of ongoing diarrheal losses, maintenance, can usually be accomplished with oral rehydration solution (ORS). For situations in which oral therapy is not acceptable (e.g., patients with severe vomiting or diarrheal stool of >10ml/kg/hr who cannot take ORS as needed or patients with additional problems that interfere with successful ORS replacement), intravenous fluids that balance the loss of electrolytes in the stool should be given for maintenance (19).

The efficacy of ORS for the treatment of diarrheal disease was established more than 25 years ago and is considered one of the most important medical advances this century in terms of the number of individuals that would have otherwise died (20). In the last 25 years, there have been minimal alterations in the basic formulation. The routine composition of ORS recommended by the World Health Organization for adults contains Na 90 mEq/L, K 20 mEq/L, Cl 60-80 mEq/L, citrate 30 mEq/L, and glucose 111 mEq/L (19). This administered preparation does not decrease the volume of diarrhea or shorten the course of the diarrheal illness. Recent attempts have been made to "improve" the solutions by modifying either the substrates used and/or the osmolality. In a number of pilot studies, solutions that have other substrates (e.g., amino acids, maltodextrins, and cereals such as rice) or a decreased osmolality (250 mOsm/L) have been shown to significantly decrease stool output and shorten the duration of the disease in certain situations (21). If on-going efficacy trials are confirmatory, this may eventually lead to a modification of the standard World Health Organization ORS formula used worldwide for all causes of diarrhea since strategies that effectively decrease the overall fluid management requirements for or ameliorate the symptoms of acute diarrhea would be important therapeutic advances for this common disease.

The use of ORS is based on the following physiologic considerations: i) Absorption of sodium from the gastrointestinal tract is coupled to that of glucose. The optimal gastrointestinal luminal ratio is ~1:1 for sodium and glucose. During secretory watery diarrhea, significant sodium absorption occurs only when it is substrate-mediated, because neutral sodium chloride absorption is inhibited by the action of the bacterial enterotoxins or by osmotic malabsorption created by damage to the intestinal villi tips, as seen in pediatric rotavirus infections. ii) Glucose absorption continues relatively uninterrupted, irrespective of the cause diarrhea; sodium will also be absorbed if concomitantly administered with glucose, irrespective of the cause. and iii) Potassium and bicarbonate are absorbed independently and do not require coupling mechanisms. Based on these observations, a standard ORS is now universally used for all dehydrating diarrhea, irrespective of the etiology or age of the afflicted individual. ORS is adequate both for rehydration and maintenance therapy.

There is an extensive literature on ORS rehydration and maintenance therapy. In brief, for all patients not severely dehydrated, the amount of volume to correct dehydration can be safely administered in a 4 to 6 hour period or on an *ad lib* basis. Interestingly,

individuals capable of *ad lib* replacement are able to more quickly correct their dehydration which is manifested by their cessation of drinking when rehydrated (18). Maintenance ORS should be given roughly in amounts equal to the amount of stool lost. If stool measurements are not possible, ORS can also be used *ad lib* if it can be made readily available. ORS is continued until diarrhea stops.

There are some important limitations to the use of ORS. Certain children who malabsorb glucose during the acute phase of illness do not absorb ORS, and a dramatic increase in stool volume results. When stool volumes are high (i.e., >10mL/kg/hr), ORS may not be adequate treatment to replace the losses. Also, among afflicted individuals that have excessive vomiting despite administering ORS in small amounts, ORS might be safely administered through a nasogastric tube. In all of these situations where it is not possible to adequately replace fluid loss per an oral route, intravenous fluids may need to be given temporally.

Despite the importance of ORS, the ideal anti-diarrheal agent has yet to be identified. It would have properties of being non-toxic, not absorbed from the gastrointestinal tract after oral administration, having no effect on intestinal motility, and effectively and rapidly blocking the chloride channel on the luminal surface in a reversible fashion (22). Unfortunately, current drugs available on the market do not possess many or all of these attributes. Therefore, research directed toward such drug development seems warranted. In view of the recent findings relevant to the clinical pharmacology and mechanism of action of SP-303, it is reasonable and important to continue to evaluate SP-303 in clinical trials for safety and efficacy in the treatment of diarrheal syndromes. One of the research priorities with SP-303 is to conduct a series of clinical trials to determine an effective oral administration regimen that involves the least amount of SP-303 drug and the least dosing interval of SP-303 to produce symptomatic relief of acute diarrhea in adults.

Table 1. Electrolyte Composition of Diarrhea Stool Caused by Different Agents

	Electrolytes (mmol/L)			
	Na^+	K^+	Cl^-	HCO_3^-
Cholera				
Child	**80**	**30**	**86**	**32**
Adult	**124**	**16**	**90**	**48**
Toxigenic *E. coli* Child	**53**	**37**	**24**	**18**
Rotavirus Child	**37**	**38**	**22**	**6**
Normal stool Child or Adult	**20-30**	**50-75**	**15**	**25-30**

II. STUDY OBJECTIVES

The primary objectives of this study are to evaluate the efficacy of orally administered SP-303 enteric-coated tablets presented in a capsule for 2 days for the symptomatic treatment of acute diarrhea syndrome among otherwise healthy adult residents of Bangladesh. Evaluation of patients receiving oral SP-303 doses of 125 mg, 250 mg, and 500 mg amounts administered every 6 hours for a 48 hour treatment course will be compared to

patients receiving matched placebo capsules every 6 hours for a 48 hour treatment course over the course of the 72-96 hour study period.

III. STUDY DESIGN

This is a prospective, randomized, double-blind, placebo-controlled, multiple-dose clinical trial to evaluate the efficacy of SP-303 for the symptomatic treatment of acute diarrhea syndrome among adult residents of Bangladesh.

Adult subjects who present to study site with a history of recent diarrhea and who fulfill inclusion criteria will be requested to participate in this study and sign an informed consent. In this study, patients will be eligible for treatment with study drug if they present to the study center with a clinical history and physical examination that are compatible with acute diarrheal disease within the last 48 hour interval and have $\geq$3 unformed (i.e., watery or soft) stools in the 24 hour period prior to study enrollment. Study subjects will undergo a complete medical history and review of organ systems, medication history, physical examination, vital signs, and collection of blood, urine, and stool specimens for laboratory and/or diagnostic evaluation. Blood will be evaluated for complete blood count and serum chemistries. Urine will be evaluated by dip-stick for protein, glucose, ketones, bile, blood, pH, and specific gravity. Conventional bacteriological methods to isolate and identify enteric pathogens will be performed on the diarrhea stool specimen. Identification of the following bacterial species are planned: *Vibrio cholerae*, *Shigella* species, *Salmonella* species, *Campylobacter* species, *Aeromonas* species, and *Plesiomonas* species. Furthermore, genetic screening of *Escherichia coli* colonies for heat-labile and heat-stable enterotoxins will be performed by DNA::DNA *in situ* hybridization. Furthermore, stools will be screened for white blood cells by standard microscopy, occult blood, and *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium* by standard light microscopy techniques.

During the screening period (that is, prior to randomization and treatment with study drug), the following information will be recorded of each patient to assess the current diarrhea episode: usual stool frequency, data and time of diarrhea onset, number of stools in the last 24 hours with each bowel movement categorized according to consistency (viz., formed, soft, or watery). Stools of a mixed form will be classified in terms of the least form category. For the purposes of this study, formed stool retains its original shape in water; soft stool assumes the shape of the container; and watery stool can be poured. Furthermore, the patient's gastrointestinal symptoms at the time of the screening visit will be recorded. The patient will be specifically asked about the following: nausea, vomiting, abdominal pain/cramps, excess gas/flatulence, urgency, tenesmus, and incontinence. In addition, the patients will grade these complaints as either severe, moderate, mild, or none.

For the purposes of randomization, patients will be assessed for the degree of disease severity in terms of degree of dehydration (none, mild, or moderate) at presentation. After determination of the disease severity, the patients will be randomized within the disease severity group to one of four treatment groups (i.e., 125 mg SP-303, 250 mg SP-303, 500 mg SP-303, or placebo administered every six hours for a total of 8 doses over the next 48 hours). Patients assigned to these treatment groups will receive the first dose of study drug after assessing clinical dehydration and effectively treating dehydration, if necessary.

The degree of dehydration at the time of initial presentation will be recorded according to earlier WHO criteria. In brief, the severity of dehydration will be classified as no signs of dehydration, signs of some dehydration, and signs of severe dehydration. No dehydration has clinical features of a normal pulse rate, normal skin turgor, good peripheral pulses,

moist mucus membranes, normal eyes in orbits, and normal sensorium that is characterized by alertness. Moderate dehydration has clinical features of marked decrease in skin turgor, tachycardia, hypotension (systolic blood pressure <100 mm Hg), dry mucus membranes, sunken eyes in orbits, and normal sensorium that is characterized by alertness, irritability, and sensation of thirst. Severe dehydration has clinical features of moderate depletion, plus absent peripheral pulses, rapid respiration, cyanosis, and altered senorium characterized by stupor or coma and lack of thirst drive. Based on the dehydration status and patient's ability to comply with the rehydration protocol, the patients will receive either fluid orally or Dhaka solution intravenously to correct dehydration due to the diarrhea syndrome according to routine standards of care. In general, oral fluids (e.g., standard WHO-recommended ORS) will be offered first and the subject allowed to drink *ad lib.* Study personnel will record all fluid intake. If the oral route of rehydration is not sufficient to correct dehydration within 2 hours or the patient continues to have stool purging, intravenous fluid will be administered.

The criteria for achieving adequate fluid homeostasis after oral rehydration in this protocol are normal skin turgor, normal orbits, moist mucus membranes, lack of thirst, normal mentation, and a urine output of 1 ml/kg/hr. Once the patient has been rehydrated and considered to be clinically stable, the subject will be administered one of the four treatments.

The surveillance period will consist of 3 days (i.e., comprising 2 days duinrg treatment and 1 additional day after cessation of study drug). Surveillance will be conducted under in-hospital conditions. Assessments will include clinical evaluation of adverse experiences, determination of stool frequency, stool volume (to the nearest one gram using a Sartorius electronic scale) and weight (to the nearest gram), stool consistency of every bowel movement, evaluation of gastrointestinal symptoms, the need for unscheduled I.V. fluids, and fluid requirements to correct or to maintain volume homeostasis after administration of the first dose of study drug.

The plan to maintain homeostasis after correcting initial dehydration at study entry or during the 3 day course of study requires that subjects be administered an equal volume of fluid for every milliliter of stool excreted. Study personnel will provide a pitcher of fluid to the study subject within 10 minutes after a bowel movement. This volume should be drunk by the subject within the next 60 minute interval, as tolerated without causing vomiting. In general, subjects will be offered the fluid of their choice (e.g., water) except when they are not able to tolerate a bland diet. Under such a condition, the subject with altered mental status will be offered standard WHO-recommended ORS which has appropriate amounts of electrolytes and glucose to replace electrolyte losses in stool. If necessary, the study subject will be administered intravenous Ringer's lactate to correct dehydration or maintain volume homeostasis if they are unable to drink sufficient amounts of fluids orally to replace stool volume losses or have altered mental status and do not drink fluid. These clinical management plans are the standard of care at this hospital and are considered appropriate to safeguard the safety of the patients. The amount of fluid required to correct dehydration or maintain volume homeostasis over the 3 day study course will be recorded, as well as the route of administration.

At the end of in-hospital surveillance, the study subject will have repeat clinical laboratory tests (i.e., complete blood count, serum chemistries, and urinalysis) performed. Thereafter, the study subject will be discharged. If the patient's medical condition at the time of planned discharge from the study warrants further therapy, it will be provided as necessary (e.g., institution of an antibiotic regimen for culture-documented enteric infection or continued hospitalization for treatment).

IV. SUBJECT POPULATION

A. Inclusion Criteria

1. Patient must sign an informed consent at the screening visit.
2. Patient must be ≥18 years to 60 years of age and have resided in Bangladesh for a minimum of 6 months preceding study entry.
3. Patient may be either male or female. However, women of childbearing age must not be breast-feeding or pregnant (as determined by a pregnancy test).
4. Patients must present to study centers with a clinical history of the abrupt occurrence of three or more unformed abnormal stools (soft or watery) within the last 48 hours in association with one or more symptoms of enteric infection (e.g., nausea, vomiting, abdominal pain/cramps, excess gas/flatulence, urgency, tenesmus, and incontinence) preceding the first dose of study medication. Also, the patient must claim to have had ≥3 unformed stools in the last 24 hour period prior to enrollment.
5. Patient must be otherwise healthy.
6. Able to take oral medication and hydration, either by mouth or intravenously.
7. Patient must be willing to comply with all the requirements of the protocol.

B. Exclusion Criteria

1. Known sensitivity or allergy to citrus fruits, black currant berries and rose hips.
2. Pregnant or nursing women.
3. Any chronic disease or medical, surgical or mental health condition which in the opinion of the investigator may interfere with the patient's ability to comply with the protocol, place the subject at increased risk, or interfere with the evaluation of the study drug.
4. The use of antimotility drugs, antimicrobials, or any other medication which might interfere with the study medication prior to the first dose of study medication. The concurrent use of any medications, which in the opinion of the investigator, may compromise the evaluation or place the study participant at increased risk.
5. Severe malnutrition.
6. Fever> 39°C (102°F) or either the need for antibiotic(s) or other prescription therapy for diarrheal disease.
7. Signs or symptoms of intestinal obstruction.
8. Have any on-going concurrent extra-intestinal disease or other extra-intestinal acute illness within 2 weeks of screening visit which, in the opinion of the investigator, may compromise the study participant's safety or evaluation of the study results.

9. Have previously been enrolled in this study, received SP-303, or any investigational drug 30 days prior to receiving study drug.

V. STUDY METHODS

A schedule of events is listed in Appendix A. The results of all evaluations will be recorded on the case report forms.

A. Screening Phase: Upon presentation to the Clinical Research and Service Centre

If an adult patient presents at the study site with a chief complaint of a recent history of diarrhea and is deemed eligible to enter the study, an informed consent form will be signed prior to the initiation of any study procedures. During the screening period, study subjects will undergo appropriate examinations and tests to establish a baseline database (e.g., stool volume, frequency, and consistency in the last 24 hour period, gastrointestinal complaints at the time of study entry after correction of dehydration, and physical examination at the time of study entry) including the collection of clinical specimens for laboratory and/or diagnostic evaluation. They will be evaluated to make certain they meet all criteria for randomization and treatment with study drugs, including assessment of clinical dehydration and correcting dehydration, if necessary. Study participants will be instructed to drink adequate fluids and will be fed a standard hospital diet during the study period.

B. Treatment Phase: 48 hours of treatment

The patients will be randomized to one of four treatment groups. The intended oral treatments for the groups are placebo, 125 mg SP-303, 250 mg SP-303, and 500 mg SP-303 every 6 hours for a total of 8 doses. It should be noted that the assignment of a Patient Number will be accomplished at the time of randomization to specific treatment group. All patients with severe dehydration will be randomized only after complete rehydration with four to six hours of intravenous fluids. Each patient will be dispensed their respective treatment medication by the Study Pharmacist who will sequentially take one capsule from the study subject's specifically assigned study drug treatment bottle at each scheduled dosing time as instructed on the label.

C. Surveillance Phase: 72-96 hours

During the two day treatment period and one to two additional days of surveillance (until diarrhea resolution) under in-hospital conditions, study personnel will monitor study participants. They will assess adverse experiences, determine stool frequency, stool volume and weight of every bowel movement, stool consistency of every bowel movement, evaluate gastrointestinal symptoms at 6 hour intervals, and assess daily fluid requirements to correct dehydration or to maintain volume homeostasis after the administration of the first dose of study drug. These monitoring activities will be recorded on appropriate case report forms. At the end of the 3 day surveillance period, repeat clinical laboratory tests (i.e., complete blood count, serum chemistry, and urinalysis) will be performed.

VI. LABORATORY EVALUATION

The following laboratory evaluations will be performed:

1. Urine Pregnancy Test: during the screening phase.

2. Automated Hematology Panel: during the screening phase but after correction of dehydration and at end of the surveillance phase, 1 ml of blood will be collected in purple top tubes for:
    - Hemoglobin (mg/dl)
    - Hematocrit (%)
    - Red blood cells ($x10^6/mm^3$)
    - White blood cells ($x10^3/mm^3$)
    - Segmental neutrophils (%)
    - Band neutrophils (%)
    - Lymphocytes (%)
    - Monocytes (%)
    - Eosinophils (%)
    - Basophils (%)
    - Platelet Count ($x10^3$ cells/μl)

3. Automated Serum Chemistry: during the screening phase but after correction of dehydration and at end of the surveillance phase, 5 ml of blood will be collected in red top tubes for:
    - Creatinine (mg/dL)
    - BUN (mg/dL)
    - Total protein (g/dL)
    - Albumin (g/dL)
    - Total bilirubin (mg/dL)
    - SGOT (AST) (U/L)
    - SGPT (ALT) (U/L)
    - GGT (U/L)
    - Alkaline phosphatase (U/L)
    - Lactic dehydrogenase (LDH) (U/L)
    - Sodium (mEq/L)
    - Potassium (mEq/L)
    - Chloride (mEq/L)
    - Glucose (mg/L)

4. Dip-stick Urinalysis: during the screening phase but after correction of dehydration and at end of the surveillance phase;
    - Protein (+ scale)
    - Glucose (+ scale)
    - Ketones (+ scale)
    - Bile (+ scale)
    - Blood (+ scale)
    - pH (units)

- Specific gravity

5. Stool Work-up: screening phase.

- Stool white blood cell microscopic screen
- Occult blood
- *Giardia lamblia* microscopy
- *Entamoeba histolytica* microscopy
- *Cryptosporidium* microscopy
- *Vibrio cholerae* culture
- *Escherichia coli* culture, plus probing of strains for putative heat-labile and heat-stable enterotoxin genes by DNA::DNA *in situ* hybridization
- *Shigella* species culture
- *Salmonella* species culture
- *Campylobacter* species culture
- *Aeromonas* species culture
- *Plesiomonas* species culture

VII. CONCOMITANT MEDICATION

Any concomitantly administered non-study medication including route, dosage and indication will be recorded on the appropriate case report.

Women will be allowed to continue taking oral contraceptives. The use of other medications during the course of the study will be discouraged, however, the Principal Investigator may allow the use of any medications that is medically indicated, in his opinion.

It should be noted that it is not known whether or not SP-303 may bind or otherwise interfere with the absorption or activity of other orally administered drugs. As a result, if there is a need to take another drug concurrently with SP-303, the subjects will be instructed not to take the medications at the same time and only to take such medications at least one hour before or after administration of study drug.

Anti-diarrheal medication (i.e., lomotil, loperamide, pepto-bismol, kaopectate, or tincture of opium) or antibiotic therapy will specifically not be allowed for the duration of the 3 day study. If anti-diarrheal medications or any other unauthorized medication are taken during the study, the subject will be terminated from the study. This action will be recorded on the appropriate case report form.

VIII. STUDY MEDICATION

A. Formulation and Packaging

SP-303. 125 mg enteric-coated tablets of SP-303 will be packaged into a gelatin capsule. The capsule strengths will be 125 mg, 250 mg, and 500 mg.

Placebo. Matched capsules lacking any SP-303 will be used as placebo.

Shaman Pharmaceuticals, Inc., the Study Sponsor, will supply bottles for each individual patient labeled with the study number, contents, dosing instructions, and precautionary

statements (e.g., "Caution: New Drug-Limited by United States Law to Investigational Use"; "Store at 20 to 30 °C" and "For Oral Use Only". The labels will be three-part labels written in English. One part will remain on the dispensing bottle. The remaining two parts will be separated from the bottle at the time of initial dispensing and will be attached to the case report form. One part of the two parts designated for the case report form will be a blinded section with the medication (SP-303 or Placebo for SP-303) and the capsule strength (0, 125, 250, and 500 mg SP-303) under an opaque, but removable cover, if it is medically indicated for the Principal Investigator to know which study drug preparation was supposedly administered to the study participant. Otherwise, this covered part of the label is to remain covered in order to maintain the double-blinded design. In the event that the Principal Investigator or the patient's attending physician deems that it is necessary to uncover the medication and strength, the Sponsor should be notified immediately. The two uncovered parts of the label (one for the bottle and one for the case report form) will have identical information.

B. Drug Administration

For patients assigned to groups receiving placebo, 125 mg, 250 mg, or 500 SP-303, the Study Pharmacist will dispense to each patient one capsule from the study subject's respective study medication bottle every 6 hours for a total of 8 doses. It will be recommended that the capsules be administered orally with fluid. The medication dispensed by the Study Pharmacist will be administered by the nurse.

Study drug should be administered at approximately 6-hour intervals for the duration of the treatment phase for each patient. If this should occur, it will be recorded in the appropriate case report form.

C. Drug Accountability

The Principal Investigator must ensure that all drug supplies are kept in a locked area with access to the study drug limited to appropriate study personnel. The Principal Investigator must maintain accurate records of the receipt of all drug shipped by the Sponsor, including date received, amount received and the disposition of all study medication. Current dispensing records will also be maintained on the appropriate case report form for each study subject. This case report form will indicate the date and the number of tablets administered by the Dispensing Pharmacist/Nurse. At the end of the study, all medication must be accounted for. Any unused study drug medication will be inventoried by the Dispensing Pharmacist and retrieved by the Sponsor, or disposed of by the Dispensing Pharmacist in accordance with local regulations and recorded in the appropriate case report form.

IX. UNTOWARD EFFECTS EVALUATIONS

A. Adverse Experiences

All symptoms or adverse experiences reported by the study subject, as well as those noted by the Principal Investigator or his designee during the treatment and follow-up period will be recorded on the Adverse Reaction Form. This form will indicate the date of onset, duration, severity and outcome. The Principal Investigator must assess the relationship of the experience to the study medication using the following criteria:

1. **Definitely associated:** the event was undoubtedly caused by administration of the study medication.

2. **No relationship:** the event is definitely related to a cause other than the study medication (a true cause was established beyond any reasonable doubt).

3. **Probable:** the event, including laboratory tests, occurs with a reasonable time sequence to administration of the drug, unlikely to be attributable to concurrent disease or other drugs and which follows a clinically reasonable response upon withdrawal of the drug (dechallenge).

4. **Possible:** the event has a timely relationship to the dose of the study medication but the direct cause of the event is unknown and other reasonably likely causes for the event may exist.

5. **Unknown:** the event does not have a timely relationship to the dose of study medication and there are no other reasonable explanations for the event.

6. **No relationship:** the event is definitely related to a cause other than the study medication (a true cause was established beyond any reasonable doubt).

B. Serious or Unexpected Adverse Events

ANY SERIOUS OR UNANTICIPATED CLINICAL EVENT, WHICH OCCURS DURING THE COURSE OF THIS INVESTIGATION, WHETHER OR NOT RELATED TO THE INVESTIGATIONAL DRUG, MUST BE REPORTED IMMEDIATELY TO SHAMAN PHARMACEUTICALS WITHIN 24 HOURS AFTER THE EVENT.

A "Serious" clinical event is defined as any event that is fatal, life-threatening, permanently disabling, a congenital anomaly, requires or prolongs hospitalization, or an overdose.

An "Unexpected" clinical event is defined as any clinical event that is not identified in nature, severity or frequency in the current Investigator's Drug Brochure.

Serious and unexpected clinical events should be promptly reported to one of the Shaman Pharmaceutical, Inc., Study Physicians within 1 day (24 hours) with any additional relevant information.

SHAMAN PHARMACEUTICAL STUDY PHYSICIANS:

Gerald Reaven, M.D.
Telephone: 415-952-7070 extension 450; or
Thomas Carlson, M.D.
Telephone: 415-952-7070 extension 473

In the event the Study Physicians cannot be reached, the following Shaman representative should be contacted and is also available by telephone Pager 24 hours a day:

Atul Khandwala, Ph.D.,
Senior Vice President Product Development
Telephone: 415-952-7070 extension 489

Telephone Pager: 888-518-1535

In addition, the investigator will prepare and send to the Sponsor and the ICDDR,B Ethical Review Committee, within a 7-day period, a written summary with a narrative description of the event. The investigator's report must contain all the available information concerning the event to allow the Sponsor to comply with regulatory guidelines. The report should include:

1. Subject's initials and number
2. Investigator's name
3. Protocol title and number
4. Subject's date of birth and sex
5. Study drug dates of administration
6. Concomitant drugs: dose, route of administration, duration of therapy and indication
7. Information regarding the adverse experience:
   - description of event
   - severity of event
   - whether or not discontinuation of study drug was required
   - dates and times of onset and resolution
   - whether hospitalization or prolonged hospitalization was required
   - whether the event is life threatening or potentially disabling
   - treatment required for the adverse experience
   - investigator's assessment of relationship of event to the study drug or possible contributing factors
   - event outcome

All fatal or life-threatening adverse events associated with the use of the study medication will be immediately reported to appropriate regulatory agencies by the Sponsor. All other serious or unexpected adverse events will be reported to the FDA within 10 working days.

Any subject who is withdrawn from the study due to an adverse event will be followed until the outcome of the event is determined.

X. END OF STUDY

A. Study Completion

The following patients will be considered to have completed the study in order to assess short-term efficacy:

i) Patients who complete 2 days of treatment and 3 days of surveillance.

ii) Patients who were terminated from the study because of refractory, severe diarrhea despite taking study drug. If a study subject is removed from the trial due to worsening or refractory, severe diarrhea, their outcome will be defined to be a **treatment failure**.

B. Early Withdrawal

If for any reason a patient withdraws or is removed from the study prior to completion of 2 days of drug treatment (8 doses), the reason for doing so will be entered on the appropriate case report form. If the patient is terminated from the trial due to intolerance to study medication, the patient will remain under medical supervision as long as deemed

appropriate by the Principal Investigator. A patient will generally withdraw or be removed from the study for any of the following reasons:

1. A serious or unanticipated clinical event precluding further therapy.
2. Subject wishes to withdraw (as stated in the informed consent, all subjects reserve the right to withdraw from the study without prejudice).
3. Investigator deems that it is in the subject's best interest to withdraw.
4. Failure to comply with the protocol requirements.
5. Subject is found to have entered the study in violation of the entry criteria.

It should be noted that when a patient is withdrawn from treatment, routine study evaluations will be performed, if possible, regardless of the reason for early withdrawal from treatment.

Patients who do not complete the treatment phase will be replaced.

XI. STATISTICAL METHODS

A. Sample Size

It should be noted that there is still limited experience in treating adult patients with oral SP-303 for the symptomatic relief of acute diarrhea. Also, in the absence of data relevant to SP-303 dose-ranging effects, it is not possible to predict efficacy outcomes on the basis of dosing equivalency.

If we assume to have 80% power to detect that SP-303 treatment would reduce stool volume between 25% and 35% of placebo treated controls at an alpha level of 0.05, we would need 72 patients to detect a 25% reduction, 53 patients to detect a 30% reduction, and 30 patients to detect a 35% reduction. Due to logistical realities and the fact a therapeutically efficacious anti-secretory agent would decrease stool volume between 30 and 35% of placebo control within a 48 hour treatment period, we intend to assess the effectiveness of SP-303 in cohorts of 35 subjects per treatment group. We intend to replace subjects who are withdrawn from the study due to any reason except for a documented treatment failure.

B. Randomization

Study subjects which have been classified according to the severity of disease based degree of dehydration at the time of presentation will be assigned to SP-303 or placebo treatment groups using a table of sequential random. Individual copies of the randomization code will be retained in a confidential fashion by Shaman's Director of Pharmaceutics.

C. Endpoints

Primary endpoints to evaluate tolerance will be assessed by comparing the number of unexpected clinical events that are definitely associated or possibly associated with study drug therapy between the placebo and SP-303 treatment groups from the start of

treatment to the end of the surveillance period. Determination of the time of onset, duration, severity, and outcome of these events will also be described and compared. Another measure of safety will include comparisons of the overall number and specific types of adverse reactions occurring during the surveillance period between treatment groups. Tolerance will also be ascertained by review of physical examination between the treatment groups from the start of treatment to the end of surveillance. Furthermore, the changes in complete blood count, serum chemistries, and urinalysis between the screening phase and at the end of surveillance will be evaluated.

The primary efficacy outcome measurements will include a comparison between treatment and placebo groups for total stool volume, the time to last unformed stool during the 48 hour treatment period, and the total number of unformed stools during the 48 hour treatment period. A significant difference is defined as $P<0.05$ (two-tailed test).

Secondary efficacy outcome measurements will include the response between treatment and placebo groups in terms of daily stool frequency, daily number of unformed stools, unscheduled I.V., and gastrointestinal symptoms at 6 hour intervals over the 3 day course of study surveillance under non-stratified conditions and stratification according to disease severity at the time of study entry, etiologic basis of diarrheal disease, and/or demographic characteristics (e.g. age group or gender). Therefore, study subjects will be evaluated according to non-dichotomous response criteria to assess efficacy at daily or 6 hour intervals compared to the baseline status at 24 hours just preceding study entry or at the time of study entry over the 3 day study. It should be noted that the baseline daily number of bowel movements and daily number of watery/soft stools will be based on the patient's history of the 24 hours preceding study entry and baseline gastrointestinal index score will be based on the patient's complaints at the time of study entry.

An **excellent response** will be defined as a $\geq$ 50% reduction in total stool weight as compared to placebo over the 3 day course of surveillance. A **good response** will be defined as $\geq$ 35% in total stool weight as compared to placebo over the 3 day course of surveillance. A **partial response** will be defined as $\geq$25% reduction in total stool weight as compared to placebo over the 3 day course of surveillance. **Poor response** will be defined as no change or <25% reduction in total stool weight as compared to placebo over the 3 day course of surveillance. It should be noted that each gastrointestinal complaint of nausea, vomiting, abdominal pain/cramps, excess gas/flatulence, urgency, tenesmus, and incontinence will graded none (score of 0), mild (score of 1), moderate (score of 2), and severe (score of 3). The gastrointestinal index score for an interval is designated by the sum of all gastrointestinal complaints. A significant difference is defined as $P<0.05$ (two-tailed test).

Furthermore, the daily stool volume and stool weight and the daily amount of fluid (milliliters) required to correct or to maintain volume homeostasis after the administration of the first dose of study drug will be compared between the treatment groups. A significant difference is defined as $P<0.05$ (two-tailed test).

D. Data Analyses

The null hypothesis will be that the SP-303 and placebo treatment groups are equivalent for efficacy. All significance testing will be performed at an α level of 0.05 for two-sided tests. The 95% confidence limits for each comparison will also be determined.

Baseline comparability will be assessed between the treatment and placebo groups, for the following variables: disease severity classification at the time of study entry, demographic characteristics (e.g., age, gender, nationality), physical examination at the

time of study entry, and stool diagnostic results performed during the screening visit. Comparison of the two groups will be done with an analysis of variance (numeric data) or a chi-square test (categorical data) under non-stratified and stratified conditions.

All analyses and summary statistics will be performed on an intent-to-treat population of all evaluable patients. All tolerance analyses will be based on subjects patients who are randomized and receive at least one dose of study drug. Study subjects terminated because they were administered or took unauthorized medications during the treatment phase will not be evaluated for safety. The following study subjects will be excluded from efficacy analyses: subjects terminated because they were administered or took unauthorized medications during the treatment phase or failed to comply with the protocol requirements. However, these subjects will be assessed for good, partial, or no responses up to the last 24 or 12 hour interval that they were monitored in the study. These criteria of evaluable subjects is consistent with an intent-to-treat analytical strategy.

Changes in adverse complaints and physical examination will be compared between treatment groups using Fisher's exact test or χ^2 test.

The primary outcome efficacy variables of total stool volume, the time to last unformed stool during the 48 hour period of treatment and the total number of unformed stools during the 48 hour period of treatment will be compared between the SP-303 and the placebo treatment groups. The time to last unformed stool will be plotted using Kaplan-Meier curves and the parameter will be analyzed using the log-rank test. Total number of unformed stools, stool volume, and stool weight will be analyzed using a non-parametric analysis of variance (ANOVA).

A number of secondary outcome efficacy variables will be assessed between the SP-303 treatment and placebo groups. Treatment responses related to effect on daily stool frequency, daily unformed stools (i.e., watery or soft stools), need for unscheduled I.V., and gastrointestinal symptoms at 6 hour intervals over the course of the 3 day study between the SP-303 treatment groups and placebo group will be assessed. They include the percentage of patients who achieve an excellent frequency response (i.e. $\geq$75% decrease in daily number of bowel movements compared to baseline), a good frequency response (i.e. $\geq$50% decrease in daily number of bowel movements compared to baseline), a partial frequency responsible (i.e. $\geq$25% decrease in daily number of bowel movements compared to baseline), and poor frequency response (i.e., $\leq$25% change in daily stool frequency compared to baseline), an excellent consistency response (i.e. $\geq$75% decrease in the daily number of watery/soft stools compared to baseline), a good consistency response (i.e. $\geq$50% decrease in the daily number of watery/soft stools compared to baseline), a partial consistency response (i.e. $\geq$25% decrease in the daily number of watery/soft stools compared to baseline), a poor consistency response (i.e. $\leq$25% decrease in the daily number of watery/soft stools compared to baseline), and excellent gastrointestinal symptom response (i.e. $\geq$75% decrease in symptom index score compared to baseline), a good gastrointestinal symptom response (i.e. $\geq$50% decrease in symptom index score compared to baseline), a partial gastrointestinal symptom response (i.e. $\geq$25% decrease in symptom index score compared to baseline), a poor gastrointestinal symptom response (i.e. $\leq$25% decrease in symptom index score compared to baseline). Excellent frequency response, good frequency response, partial frequency, poor frequency response, excellent consistency response, good consistency response, partial consistency response, poor consistency response, excellent symptoms response, good symptoms response, partial symptoms response, and poor symptoms response will be compared between treatment groups and placebo group using Fisher's exact test. Also, these responses will be compared under stratified conditions according

to disease severity (i.e., either according to the number of unformed stools in the preceding 24 hour period or according to clinical dehydration) at the time of enrollment, the presumptive etiologic basis of the diarrheal disease, and/or selected demographic characteristics.

The time interval until first formed stool during the treatment period will be summarized using Kaplan-Meier curves and compared between treatment groups using the log rank test and the percentage of patients who are determined treatment failures will be compared between the SP-303 and placebo treatment groups using a chi square test.

Furthermore, additional secondary parameters of efficacy will be evaluated. They include the daily stool volume and stool weight and the daily amount of fluid (milliliters) required to correct or to maintain volume homeostasis after the administration of the first dose of study drug between the SP-303 and placebo treatment groups. Differences will be assessed by using a chi square test.

XII. INFORMED CONSENT

Signed informed consent will be obtained from each subject prior to entering the study. Subjects will be informed of the nature of the study and of any potential risk in participating in the study. The Informed Consent will be provided in English and Bengali. The Principal Investigator will ensure that Informed Consent is obtained from each subject prior to study entry in accordance with current regulations. The signed consent forms will be retained by the Principal Investigator with the study records.

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Budget For SP-303

EFFICACY OF SP-303 IN ADULTS WITH ACUTE WATERY DIARRHEA	
Description	
Duration: 18 months	
Subjects: 155 adults with acute watery diarrhea.	
Research ward: Each subject for 4 days.	
1. Personnel	US Dollar
Dr. George Fuchs (15%)	nil
Dr. H. Ashraf 40%	8,400
Dr. N. H. Alam 15%	4,650
Study Physician 100% - 2	9,500
Study Nurse 100%-6	8,500
Sr. Health Assistant - 2	8,000
Secretarial service 40%	2,850
2. Research Ward	
Patient hospitalization	15,500
Lab tests	25,740
3. Miscellaneous	
Local Transport	3,100
Xerox, publication, printing	2,800
Hospital supplies	4,200
Office supplies	2,000
Communications (fax, international mail, etc.)	4,000
Travel (to present study results at international conferences)	9,000
Capital Expenditure	
(Computer & other accessories)	7,500
Microscope	2,000
Direct Costs	117,740
Indirect Costs (31%)	36,499
Total Costs	**$154,239**

Appendix A: Schedule of Events

	Screening	Study Day		
		1	2	3
Informed consent	X			
Selection criteria	X			
Stool volume and weight	X	X	X	X
Stool frequency and consistency	X	X	X	X
Gastrointestinal symptoms	X	X	X	X
Correction of dehydration	X			
Study drug		X	X	X
Fluid requirements for homeostasis		X	X	X
Need for unscheduled i.v.		X	X	X
Assessment of adverse reactions	X	X	X	X
Physical exam plus weight	X	X	X	X
Clinical laboratory:				
Pregnancy test (all women)	X			
Complete blood count	X			X
Serum chemistries	X			X
Urinalysis	X			X
Stool evaluation	X			

Abstract Summary for the Ethical Review Committee

1. This protocol aims to evaluate the efficacy of a new antisecretory agent Provir ™ (SP-303) in the treatment of acute watery diarrhoea in adults.

   Provir ™ (SP-303) is an agent isolated from a plant of the family Euphorbiaciae. The red, viscous latex from this plant is widely used for its medicinal properties by rural and urban people of Central and South America. Its most common use is in diarrhoea and stomach ulcers. No side-effects have been reported by indigenous populations using the drug. SP-303 is the biologically active form and, chemically, a heterogeneous phenolic polymer.

   SP-303 has been shown in a number of studies to inhibit chloride secretion in response to bacterial enterotoxin, thus reducing cholera toxin-induced secretory diarrhoea in animal models. The toxicity of SP-303 has been studied in several animal species. The drug was well tolerated at oral doses as high as 500 mg/kg/day for 14 days and 200 mg/kg/day for 30 days in rodents and dogs.

   The safety of SP-303 in humans has been extensively evaluated in several clinical studies involving healthy volunteers and patients. The drug was well tolerated even in paediatric patients with no significant side effects.

   This drug has been proved to be an effective antisecretory agent in multiple studies. In a few studies involving diarrhoeal patients, the drug has also been shown to be effective in reducing diarrhoeal illness. It is therefore expected that this drug used as an antidiarhoeal agent will reduce the severity and duration of diarrhoeal illness in our population with diarrhoea .

2. The investigations will not cause any known risk to the patients.

3. Informed consent will be obtained from all patients.

4. A short interview will be taken to obtain the clinical history.

5. There will be immediate benefit to the patient, by investigation, results, specific treatment, constant monitoring and appropriate treatment measures.

6. Venous blood will be taken for electrolyte, CBC, BUN, creatinine, liver enzymes, platelets. Rectal swab/stool will be obtained for microbiological tests. Stool will be taken for microscopy.

7. Patients' records will be necessary for data analysis.

8. Patients will have to stay in the hospital for 3-4 days.

CONSENT FORM

" A double-blind, randomized, placebo-controlled, dose-response study to assess the efficacy of SP-303 (Provir ™) in the treatment of acute diarrhoea."

(Statement read and clearly explained to the patients when consent is obtained.)

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is carrying out research to find out a simple, effective and inexpensive treatment for diarrhoeal diseases. At present, you are suffering from acute diarrhoea, the management of which includes replacement of fluid and electrolyte loss with oral rehydration therapy (ORT) and appropriate feeding during and after diarrhoea. But as ORT does not reduce the severity and duration of diarrhoea, demand for effective antidiarrhoeal drugs are high. Still, we do not have any effective antidiarrhoeal drug.

Provir ™ (SP-303) is a new antidiarrhoeal drug that is expected to reduce diarrhoeal disease severity and disease duration. We request you to participate in this study. So, if you agree to participate into the study, you will receive either Provir ™ at daily doses of 0.5, 1.0 or 2.0 grams or placebo for 48 hours. In addition you will get ORT and proper feeding. You have to stay in our hospital until diarrhoea stops. Two teaspoons (6 ml) of blood as well as stool and urine will be collected on admission and after 3 days and sent to the laboratory for proper examination. The results of the investigations will be used to evaluate the effect of treatment. You will be discharged after diarrhoea stopped and other necessary treatment completed.

Taking part in the study totally depends upon your own decision. If at anytime you wish to withdraw yourself from the study you are free to do so without any obligation. Even then you will get the standard treatment in this hospital. If you are willing voluntarily to join in this study, please sign or give your left thumb impression.

_____ _____

Signature of Investigator

Signature/Left Thumb Impression of the patient

Witness _____

Date _____