Attachment 1.

Date 28 March 1988

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator Dr. A. Salam Dr. M. Bennish

Trainee Investigator (if any) _____

Application No. 88-011

Supporting Agency (if Non-ICDDR,B) _____

Title of Study Short course ciprofloxacin in the treatment of shigellosis

Project status:
(X) 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
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.

(PTO)

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

28-3-88

Principal Investigator

Trainee

88-011
28/3/88

Section 1: Research Protocol

1. Title: Short Course Ciprofloxacin in the Treatment of Shigellosis

2. Principal Investigators: Dr. M. Bennish

International Research Associate,
International Centre for Diarrheal
Disease Research, Bangladesh

Dr. A. Salam
Chief Physician
Dhaka Treatment Centre
ICDDR,B

3. Starting Date: As soon as approval is granted

4. Completion Date: Three years after initiation of the study.

5. Total Direct Cost: US$118,080

6. Scientific Program Head: This protocol has been approved by the Clinical Sciences ~~Working~~ Division

Signature of Programme Head: Shakalah

Date: 28.3.88.

7. ABSTRACT SUMMARY

This study will examine whether a single dose of 1 gram of ciprofloxacin, or two doses of 1 gram of ciprofloxacin given on two consecutive days, are as effective as 500 mg of ciprofloxacin given in ten 12 hourly doses over five days in the treatment of shigellosis in adults. The latter dose of ciprofloxacin has been shown more effective then ampicillin in the treatment of adults with shigellosis in a study that we have recently completed here at the ICDDR,B. Ciprofloxacin, unlike ampicillin, is active *in-vitro* against virtually all *Shigella* isolates tested here at ICDDR,B. We will enroll 40 patients eligible for analysis in each treatment group - 120 eligible patients in all. Initial enrollment will be approximately 160 patients in order to achieve the 120 patients who are required for analysis. Patients eligible for study will be males 18 to 59 years of age with less than a 72 hour history of dysenteric illness, who have not had prior, potentially effective therapy for their current illness, and who give informed consent to join the study. Treatment will be randomized and double blinded. Patients will be hospitalized for six days. Histories will be taken, and physical examinations and cultures of stool (or rectal swabs) will be performed daily. Patients will be requested to return for follow-up examination and culture of a rectal swab sample approximately 10 days after discharge. Patients will be considered clinically cured if on study day 5, the last treatment day, they are afebrile, have no unformed stools, and have three or less soft or formed stools. Patients will be considered bacteriologically cured if *Shigella* is not isolated from a stool or rectal swab culture on day three, or on any subsequent study days.

8. Reviews

i. Ethical Review Committee

ii. Research Review Committee

iii. Director's signature and remarks, if any:

SECTION 11 - RESEARCH PLAN

A. INTRODUCTION

1. Objective

a. The objective of this study is to determine if a short course of ciprofloxacin, either one dose of 1 gram, or two doses of 1 gram on consecutive days, is as effective as standard therapy of 10 doses of 500 mg given 12 hourly for five days, in the treatment of adult male patients with shigellosis.

b. To determine the selective antibody response to A and B subunits of Shiga toxin.

c. To determine if sequential levels of C-reactive protein and the zeta-sedimentation ration correlate with effectiveness of therapy as determined by clinical response.

2. Background

Shigellosis remains a major cause of morbidity and mortality in Bangladesh (Bennish). In our own Dhaka Treatment Centre it remains the major identifiable infectious cause of death (annex 1). Resistance to the standard antimicrobial agents used in its treatment is now common (annex 2), and has required us to adopt nalidixic acid as the drug of choice for the empiric therapy of this infection (Salam, Bennish submitted for publication). Resistance to nalidixic acid has now become common in certain areas of Bangladesh and India (Munshi, Panhorta) and such resistance can be expected to spread to other areas of the country in the future. There remains a need therefore to identify alternative antimicrobial drugs, and antimicrobial regimens, for use in the treatment of this disease.

Ciprofloxacin is a member of a new generation of fluorinated quinolones which include (among the agents that have been marketed) enoxacin,

norfloxacin, ofloxacin, and pefloxacin (Neu). These agents, like nalidixic acid and oxolinic acid, (members of the first generation of quinolones), exert their anti-bacterial activity by inhibiting bacterial DNA gyrase, which is responsible for the coiling of DNA, and which in turn is necessary for the integrity of the DNA (Hooper).

Ciprofloxacin and other members of the second generation of quinolones possess several advantages over the earlier quinolones, nalidixic acid and oxolinic acid. These advantages include; 1). their broader spectrum of activity - not only are they active against almost all of the Enterobacteriaceae, which nalidixic acid had only limited activity against, but they also have activity against *Pseudomonas* sp. and gram-positive cocci (Sanders); 2). serum levels after oral or intravenous administration, in relation to the minimum inhibitory concentration of the organism which they are being used, are much higher than with the first generation quinolones (Bergan); 3). the rate of spontaneous mutation is much lower (Hooper).

Ciprofloxacin is perhaps the most widely studied of the new generation of quinolones. A search of the medical literature through the end of 1986 identified 2,461 publications on this new agent. Ciprofloxacin has a number of advantages over other of the new quinolones - primary among these is its greater potency against the gram-negative organisms for which these agents are likely to be used most commonly. Ninety percent of *Shigella* are inhibited by $\leq$ 0.007 micrograms (Sanders); mean peak serum concentrations after a standard 500 mg dose have, in most studies, been in the 1.5 to 3 microgram range, a concentration 350 times greater than the MIC (Bergan). For comparison, norfloxacin is approximately 10 times less potent against *Shigella* (Diaz-Enciso, Reeves, Goosens). Ciprofloxacin shares with the other second generation quinolones a relatively long serum half-life (Davis,

Hoffken). Most studies have shown that the half life of ciprofloxacin is in the range of 3 hours (Wise). This has allowed for the relatively long intervals between dosing - with dosing usually being done on an every 12-hour, or an every 24-hour basis.

About half of an oral ciprofloxacin dose is excreted unchanged in the urine (Bergan). This results in very high urine concentrations, and hence the initial use of ciprofloxacin was in the treatment of urinary tract infections (Goldstein). Some of the the remaining 50% of an oral dose is probably metabolized in the body to active and inactive metabolites that are then excreted in the urine (Bergan). There is also the suggestion from some studies that there is an active excretion process into the gut, probably via direct transport across the intestinal mucosa rather than through a biliary pathway thus resulting in very high levels of active drug in the lumen of the large bowel (Segreti). This occurs in spite of the fact that the majority (average from different studies is 70%) of an orally administered dose is absorbed in the upper gastrointestinal tract (Bergan).

Ciprofloxacin penetrates well into tissue (Wise). Concentrations in blister fluid are almost always in excess of levels in the serum (Wise). The same is true for muscle and lymphoid tissue (Wise). More remarkably ciprofloxacin penetrates well into tissues that most antibiotics have difficulty penetrating into; levels in non-inflamed cerebrospinal fluid are 20% of peak serum levels after a 500 mg oral dose; levels in inflamed meninges are 40% of peak serum levels (Wise, Wolff). Levels in bone, a tissue where achievable levels with most antibiotics are often sub-optimal, are 20-30% of serum levels with ciprofloxacin (Wise). This latter finding has resulted in a revolution in the treatment of osteomyelitis, especially chronic osteomyelitis, as there are few oral agents that both are *in-vivo* active against the majority of microbial pathogens that commonly cause

osteomyelitis, and that are also reach adequate levels in bone. Thus previously most patients with osteomyelitis have required prolonged hospital stays for the administration of expensive, and often relatively toxic, parenteral antibiotics. The availability on the market of the new quinolones, which can be given orally, are active against the majority of organisms causing osteomyelitis, and reproducibly achieve high levels within bone, has thus resulted in a marked change in the management of osteomyelitis in those countries where ciprofloxacin is available.

Concentrations of ciprofloxacin in stool after administration of 500 mg 12 hourly for five days have been in the range of 100 to 500 ug/g of stool (Segreti). These levels are over a thousand-fold in excess of the MIC's of most enteric pathogens. Although these levels result in the eradication of most of the aerobic gram-negative gut flora during the duration of treatment, in numerous studies this has not resulted in the overgrowth of either fungi or *Enterobacteriaceae* resistant to ciprofloxacin (Rozenberg-Arska, Dekker, Brumfitt, Shah). This most likely is because ciprofloxacin is not active against the gut anaerobic flora, or against the gut aerobic streptococci, both of which play a prominent role in "colonization resistance" in the gut (Young). Colonization resistance is a process that has been elucidated by a Dutch team of microbiologists and oncologists, and refers to the effect that the gut anaerobic flora and streptococci have in limiting colonization by anaerobic bacteria (van der Waaij, Guiot). Drug regimens that eliminate this anaerobic flora predispose to subsequent colonization with an aerobic flora that often is multi-resistant (Young).

Another tissue site that ciprofloxacin penetrates well is white cells. This ability to penetrate white cells well has proven of benefit in the use

of ciprofloxacin for the treatment of *Salmonella* infection, an infection in which the bacterium is often located within white cells (Bryan).

One last advantage of ciprofloxacin is that it can also be administered intravenously (Drusano). Thus in patients who are severely ill and unable to take oral medications, the drug can be administered parenterally.

Adverse reactions to ciprofloxacin serious enough to warrant stopping the drug are extremely rare (Arcieri). The most common mild adverse reactions to ciprofloxacin involve the gastrointestinal tract and the central nervous system. Gastrointestinal adverse effects include nausea, vomiting and diarrhea. Central nervous system side effects include dizziness, headaches, tremors and restlessness. In many of the patients with reported side effects it was often difficult to discern whether ciprofloxacin was the drug responsible for the side effect, as many patients were also on concurrent medicine, such as theophylline (Arcieri). All quinolones, including ciprofloxacin, can at very high doses cause arthropathy in immature animals of a number of different species (Ingham). This effect was first identified with the administration of nalidixic acid to beagle pups, and since has been found to occur with the newer quinolones (Ingham). The implications of this for man are unclear, as this complication has not been found to occur in man, even when nalidixic acid has been given to children (Schaad).

Ciprofloxacin is a relatively inexpensive drug, especially when compared to other groups of drugs which have such broad spectrum activity, the third generation cephalosporins. The price of ten 500 mg ciprofloxacin tablets (a standard five day course) in the United Kingdom is 7 pounds 50 pence (package insert that accompanies the drug). This is remarkably inexpensive for a drug that has been recently licensed and is still on patent, and is less than 10% of the cost of a five day course of a third

generation cephalosporin such as ceftriaxone or moxalactam. If one were to include the cost of administration of a third generation cephalosporin, which must be given parenterally, the cost differential would be even greater (Barriere, Quintiliani).

Ciprofloxacin is licensed for use in the United States, the United Kingdom, West Germany, and most other developed countries. The major clinical uses of ciprofloxacin, some of which have been commented on above, include treatment of bone and joint infections, urinary tract infections, sexually transmitted diseases, bacterial diarrhea, and lower respiratory tract infection. In bone and joint infections, urinary tract infections, sexually transmitted diseases and bacterial diarrheas ciprofloxacin is likely to be active against almost all of the common, as well as the uncommon, microbial pathogens at these sites. There are unlikely to be other oral agents with such a broad antibacterial spectrum, and with such a wide therapeutic margin. The case of lower respiratory tract infections is somewhat different, as there are other agents with a more narrow spectrum that are likely to be effective against most of the common pathogens. The use of ciprofloxacin for lower respiratory tract infections is thus likely to be restricted to patients who are infected with organisms for which ciprofloxacin is especially effective, such as cystic fibrosis patients, who are often infected with a variety of *Pseudomonas* sp. and methicillin resistant *Staph aureus* (Hodson).

Our recently completed study of ciprofloxacin for the treatment of shigellosis is the largest study of the use of one of the newer quinolones for the treatment of shigellosis (Bennish, Salam unpublished data). In this study, which has not yet been published, we found that 57 (97%) of 59 patients who completed five days of therapy with ciprofloxacin had either

complete resolution or marked improvement in their illness compared to 22 (88%) of 25 patients treated with ampicillin who were infected with a strain of *Shigella* sensitive to ampicillin (NS), and only 15 (43%) of 35 patients who were infected with a *Shigella* strain resistant to ampicillin and who were initially treated with ampicillin (P <0.001, ampicillin resistant group vs. ciprofloxacin or ampicillin sensitive group). Patients treated with ciprofloxacin had *Shigella* eradicated from there stool significantly faster; at the end of day one of therapy only 2 (3%) of 62 ciprofloxacin treated patients were still excreting *Shigella* compared to 5 (19%) of 26 patients treated with ampicillin who were infected with an ampicillin sensitive strain (P = 0.003) and 16 (43%) of 37 patients receiving ampicillin who were infected with an ampicillin resistant strain (P <0.001). Two earlier smaller studies, conducted in Central Africa, compared norfloxacin and oxolinic acid to the older quinolone nalidixic acid for the treatment of shigellosis (Rogerie, De Mol). Both of those studies found results similar to ours; the new quinolone achieved a rapid bacteriologic and clinical response. Other, large scale studies of the ciprofloxacin for the treatment of diarrhea have been conducted in travelers (Ericsson).

Increased compliance and decreased cost are the major reasons for attempting to determine whether a short course of therapy is effective in the treatment of shigellosis. Although problems of compliance are common in developed countries, the problem is even more acute in less developed countries, where there is, for many reasons, a tendency for practitioners to prescribe or sell less than the standard course therapy (Hossain). The problem of cost is also more acute in less developed countries - a course of therapy that costs even $2 (let alone 7 pounds 50 pence) is a considerable expense for most families in this country, and is actually prohibitively expensive for many of the poorest families. Reducing the amount of

medication required by 80, or 60%, would represent a considerable and important saving to many persons.

Finding effective treatment regimens for shigellosis that can be easily complied with is a major public health concern in Bangladesh. In many areas of Bangladesh dysentery due to shigellosis is one of the major, (if not the major) cause of childhood mortality (Ronsmans, Bennish submitted for publication). Current treatment regimens depend on co-trimoxazole, ampicillin, or nalidixic acid. Resistance to all three of these agents, including nalidixic acid, is becoming more common, and with our finding that ciprofloxacin is effective in the treatment of this infection it is likely that it will become one (if not the) drug of choice for shigellosis in the [illegible] resistant strain ($p < 0.001$). Two earlier smaller studies, conducted in Central Africa, compared norfloxacin and oxolinic acid to the older quinolone nalidixic acid for the treatment of shigellosis (Rogerie, De Mol). Both of those studies found results similar to ours; the new quinolone achieved a rapid bacteriologic and clinical response. Other, large scale studies of the ciprofloxacin for the treatment of diarrhea have been conducted in travelers (Ericsson).

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have adequate levels of drug in the gut lumen, and it is unlikely that either of the two drugs, which were given parenterally, achieved luminal levels that were in excess of the MIC of the *Shigella* that were being treated.

The other three studies of short course therapy have all examined the usefulness of tetracycline (Stoker, Lionel, Pickering). The first of these studies was conducted in the early 1960's in Cyprus and compared an oral mixture of streptomycin and sulphadimidine with a single dose of 2.5 g of tetracycline (Stoker). The authors found that the bacteriologic cure rate was 82% in 33 patients who received the streptomycin-sulphadimidine combination, compared to 90% in patients who received tetracycline. Although this study and its methods are somewhat dated by now (the short course therapy is referred to as "stosstherapy", after a practice of giving a single massive dose of vitamin D to patients with rickets, the method by which it worked was referred to as "therapia sterilisans magna", the price of the 250 mg of tetracycline was considered quite expensive at 12 shillings, and patients were hospitalized for an average of 13 days in the strep - sulpha group compared to "only" 9 days in the tetracycline group), the conclusion, that a short course of therapy was as effective as a longer course of therapy in achieving bacteriologic cure, seems relatively solid.

The second study of short course tetracycline therapy was conducted in Sri Lanka and compared a single 2.5 g dose with a five day course of 250 mg q 6 h. There were 39 patients in each group, and the clinical and bacteriologic response in the two groups was similar. The third study was an open study of 2.5 g of tetracycline in 26 symptomatic and 16 asymptomatic *Shigella* infected American students attending a summer university course in Mexico (Pickering). In this open study patients infected with strains of *Shigella* that were resistant to tetracycline had clinical and bacteriologic

responses that were similar to patients that were infected with sensitive strains. Given the lack of a control group, it is difficult to asses the overall impact of therapy.

There is reason to think that ciprofloxacin will be even more effective than either ampicillin or tetracycline when given for a short course. In our recently completed study we found that after 2 doses of 500 gm ciprofloxacin 60 (97%) of patients had *Shigella* eradicated from their stools, compared to 21 (81%) of 26 patients who had received 4 doses of 500 mg ampicillin and were infected with a strain of *Shigella* that was sensitive to ampicillin. Only 21 (57%) of 37 patients receiving ampicillin who were infected with a *Shigella* strain resistant to ampicillin had *Shigella* eradicated from their stool after the first day of therapy ($P < 0.001$) when compared to ciprofloxacin).

The most likely explanation for this rapid response to ciprofloxacin is the fact that the drug achieves such high serum levels in relation to the MIC of the organism. Over 90% of the *Shigella* strains isolated from patients in our previous study were inhibited by 0.015 micrograms of less of ciprofloxacin. Mean peak serum levels in patients from our previous study were 1.44 micrograms, or approximately 100 times the MIC of 90% of the isolates. Even mean trough levels, taken 11.5 hours after a ciprofloxacin dose, were 0.64 micrograms, approximately 40 times the MIC. After a 1 gram dose of ciprofloxacin mean peak serum levels should be in the range of 5 micrograms; given the 3 hour half life of the drug, after a single 1 g dose serum levels should remain above the MIC of 90% of the organisms for at least 8 half-lifes, or 24 hours. Such peak drug levels are still well within the therapeutic, and non-toxic range. Drug levels in the stool, given that 30% of the drug is not absorbed and a portion of that which is

absorbed is excreted into the gut lumen, should even be higher. These pharmacologic considerations, when taken as a whole, and combined with our experience in our recently completed study of ciprofloxacin in the treatment of shigellosis, provide very strong evidence for thinking that a short course of therapy is likely to be effective.

Shiga toxin is a protein that is produced in large quantities by *Shigella dysenteriae* type 1 and by certain serotypes of *Escherichia coli*, and in smaller amounts by a variety of other enteric pathogens, including all *Shigella* species (Keusch 1972a, Catney). It has a variety of effects in different animal models, including the limb paralysis and death after injection into susceptible animals, (hence its description as a neurotoxin), the induction of fluid secretion when infected in rabbit ileal loops (entertoxicity) and when applied to a variety of different continuous cell lines in culture, the death of the cells (cytotoxicity) (Keusch 1972b, Keusch 1975). These biologic properties had all been described prior to the purification of the toxin, hence Shiga toxin was variously known as Shigella neurotoxin, cytotoxin, and enterotoxin. With the subsequent purification and characterization of the toxin it is now known that all of these different biologic activities are due to the same protein, which is composed of a single, large A chain and multiple B chains which associate non-covalently to form the holo-toxin. The molecular weight of the holo-toxin is approximately 64,000, comprising an A subunit of 32,000 molecular weight, and 5 B subunits of approximately 6,000 molecular weight. The A chain is known to inhibit protein synthesis in vitro by catalytically inactivating the eukaryotic 60S ribosomal subunit, and it is thought that the B subunit is responsible for binding to a glycoprotein receptor (Donohue-Rolfe). In this respect (a larger A subunit responsible for the action of the toxin, and a smaller B subunit which binds the toxin to a

receptor) Shiga toxin resembles a number of other biologic toxins, such as cholera toxin.

Infection with *S. dysenteriae* type 1 is known to reproducibly produce an antitoxin response in man (Keusch 1976). Small scale studies of patients infected with *Shigella flexneri* and *Shigella sonnei* have shown that infection with these serotypes of *Shigella* also results in an antitoxin response. Despite the evidence of an immune response to the toxin, the role that toxin actually plays in man remains incompletely defined. The recent discovery that *E. coli* 0157:H7 produces large amounts of Shiga toxin *in-vitro*, and that infection with this organism, as with *S. dysenteriae* type 1, is associated with a relatively rare complication, the hemolytic-uremic syndrome, provides strong presumptive evidence that Shiga toxin is involved in the pathogenesis of this complication (Karmali, Anonymous, Lancet editorial).

The recent isolation, purification and amino acid sequencing of the two subunits of Shiga toxin make it possible for us to distinguish antibody response in man to each of the two subunits (Seidah). If, as is likely, it is found that Shiga toxin does play a role in the pathogenesis of HUS or other complications of *S. dysenteriae* type 1 infection, it will be important when developing vaccines to know the response to each of the two subunits. Having a defined group of patients with known *Shigella* infection under our care from early in their illness until at least day 17 of illness will allow us to do this. Determining the outcome of therapy in patients with dysentery is somewhat less straight forward than is the case for watery diarrhea, where the volume of stool output becomes the critical determinant. The clinical features of dysentery are varied, and some symptoms might persist even when others have resolved. Our experience from our previous

studies of dysentery is that this is the case. When patients were treated with effective therapy, fever was the first of the symptoms to resolve, while evidence of colitis, such as WBC's on microscopic examination of stool, persisted. It might be of use to have alternative measures of the effectiveness of therapy, especially of the persistence of an inflammatory colitis, and C-reactive protein (CRP) and the zeta-sedimentation ratio are two safe, inexpensive and easily performed measures of the intensity of inflammation. CRP is made in the liver in response to an inflammatory stimulus (Bennish, 1984a). It has been used to detect disease, as well as to monitor progression of disease. The erythrocyte sedimentation rate (ESR) has served a similar function (Sox). The zeta sedimentation ratio (ZSR) is

syndrome, provides strong presumptive evidence that Shiga toxin is involved in the pathogenesis of this complication (Karmali, Anonymous, Lancet editorial).

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Resistance to nalidixic acid has now appeared in parts of Bangladesh, and in the future resistance is likely to spread. Ciprofloxacin is a second generation quinolone which is very active in vitro against *Shigella*. Clinical and laboratory experience to date has shown that development of resistance among *Enterobacteriaceae* to this drug occurs much less commonly than in the case of nalidixic acid and the earlier generation of quinolones. In a recently completed study of we have shown that ciprofloxacin is as, or more effective, than ampicillin, in treating *Shigella* infection when both were given for a standard five day course. Clinical, and pharmacologic results from that, and other studies, provide strong evidence for believing that a shorter course of therapy would be equally as effective in the treatment of shigellosis. A shortened course of therapy would have the advantage of increased patient compliance and decreased cost, important considerations when dealing with a public health problem of this magnitude.

B. SPECIFIC AIMS

The specific aims of this study are:

1. To determine whether a single 1 gram dose, or two doses of 1 gram on consecutive days, are as effective in treating adults with shigellosis as a standard course of 500 mg 12 hourly for five days.

2. To determine the pharmacokinetics of ciprofloxacin in patients with shigellosis; specifically to determine the serum half-life of the drug in patients with shigellosis, to determine peak and trough levels after both a 500 mg and 1 g dose, and to determine the level and duration of enteric excretion in patients who receive a short course of therapy.

3. To determine if the ZSR and CRP are useful alternative measures of the effectiveness of therapy, particularly the resolution of the inflammatory colitis that may persist after bacterial cure is achieved.

C. METHODS OF PROCEDURE

1. Patient Selection.

a. Entry criteria

Patients eligible for the study will be males 18 through 59 years of age who come as outpatients to our Dhaka Treatment Centre with a history of bloody dysentery of less than 72 hours duration, and who have more than 20 white blood cell per high power field on microscopic examination of the stool. Patients will be selected in a "convenience" manner; that is we will attempt on a space available basis to enroll all patients who meet the entry criteria, but we will not necessarily offer enrollment to all patients coming the the Treatment Centre during this time who meet the eligibility requirements. Signed informed consent will be requested from all patients prior to their entry into the study.

b. Reasons for exclusion from study

Patients who would otherwise be eligible for inclusion in the study will be excluded if they; i. Have taken for their current illness an antimicrobial agent that is potentially effective against *Shigella*. In actual practice this will mean that patients who have taken co-trimoxazole, ampicillin, or nalidixic acid prior to coming to the Treatment Centre will be excluded from the study, while patients who have taken tetracycline, furazolidone, or metronidazole will be eligible for the study; ii. Patients who have evidence of infection in addition to their gastrointestinal infection will be excluded; iii. Any known allergy to the study drug, ciprofloxacin, or to other quinolones.

2. Treatment Regimen

Patients will be randomized in equal numbers to receive either 1 g ciprofloxacin as a single dose, 1 g ciprofloxacin on two consecutive days at a 24 h interval, or 500 mg ciprofloxacin 12 hourly for 10 total doses for five consecutive days.

3. Randomization

Patients will be assigned consecutive study numbers upon enrollment into the study, and will be given therapy with a regimen that has been pre-assigned to that study number. Assignment of therapy will be done prior to the initiation of the study using a random number table and a block randomization method. Medication will be prepared in capsule form, and packaged in blister packs. The drugs for all four treatment regimens will be prepared in identical appearing blister packs; patients receiving short course therapy will have an inactive capsule in place of drug for those doses for which no active drug is to be given. Therapy will therefore be double blinded.

4. Clinical Evaluation and Laboratory Studies

Patients will be hospitalized in the adult study ward of the Dhaka Treatment Centre for six days, and will be requested to return for follow-up 10 days after discharge.

The following will be the schedule for clinical and laboratory evaluations:

Admission:

a. Complete history and physical examination.

b. Stool microscopic examination.

c. Culture of stool to identify *Shigella* sp., *Salmonella* sp., *Vibrio* sp., *Campylobacter jejuni*, *Plesiomonas shigelloides* and *Aeromonas hydrophilia*.

d. Culture of rectal swab sample to identify *Shigella* sp.

e. Complete blood count and differential count (0.5 ml whole blood).

f. Serum electrolytes, creatinine, SGPT, total bilirubin, C-reactive protein (2.5 ml whole blood), ZSR (0.1 ml whole blood).

g. Blood culture (3.0 ml whole blood).

h. Measurement of antibody response to the A and B sub-units of Shiga toxin (2 ml of whole blood).

i. Urinalysis

Day 1.

a. History and physical examination.

b. Vital signs every 4 hours.

c. Enumeration and characterization of each stool.

Definitions - Stools will be characterized as either watery (can be poured); soft (takes the shape of the container in which it is placed) and formed (retains it's own shape). Each stool will also be characterized as having gross blood and mucous according to the following scale; gross (majority of stool). moderate (equal amounts of fecal material) and trace (streaks of blood and or mucous).

d. Determination of peak drug level ninety minutes after the first dose of medication (2 ml of whole blood).

e. Rectal swab and stool culture for *Shigella*.

Day 2.

a. History and physical examination.

b. Vital signs every four hours.

c. Drug levels just prior to, and 90 minutes, 4 hours and 11.5 hours after 8 am dose of antibiotics (8.0 ml of whole blood required).

d. Enumeration and characterization of stools.

e. Rectal swab and stool culture for *Shigella*.

f. Stool sample for determination of drug level. (1st sample after 8:30 a.m.)

g. CPR and ZSR determination (0.5 ml whole blood).

Day 3.

a. History and physical examination

b. Vital signs every four hours

c. Enumeration and characterization of stools.

d. Rectal swab and stool culture for *Shigella*.

e. Microscopic examination of stool.

f. Stool sample for determinants of drug level. (1st sample after 8:30 a.m.)

Day 4.

a. History and physical examination.

b. Vital signs every four hours.

c. Culture of rectal swab and stool samples for *Shigella*.

d. Enumeration and characterization of stools.

e. Stool sample for determination of drug level.(1st sample after 8:30 a.m.)

Day 5.

a. History and physical examination.

b. Vital signs every four hours.

c. Culture of rectal swab and stool samples for *Shigella*.

d. Enumeration and characterization of stools.

e. Determination of trough ciprofloxacin 30 minutes prior to last dose (2 ml whole blood).

f. Determination of antibody response to A and B sub-units of Shiga toxin (2.0 ml of whole blood required).

g. Measurement of CRP and ZSR (0.5 ml of whole blood).

Day 6.

a. History and physical examination.

b. Vital signs every four hours.

c. Enumeration and characterization of stools.

d. Culture of rectal swab and stool sample for *Shigella*.

e. Discharge at the end of the study day.

f. Stool sample for determination of drug level (1st sample after 8:30 a.m.)

10 Post Discharge Visit

a. Culture of rectal swab for *Shigella*.

b. Determination of antibody response to A and B sub-units of Shiga toxin (2.5 ml of whole blood required).

c. Measurement of CRP and ZSR (0.5 ml whole blood).

A summary of the information that will be obtained during the study is provided in the form for computer entry of the data, a copy of which is attached (Annex 3).

5. Sample size determination

We require a sample size of 40 evaluable patients in each treatment group in order to test with a power of 80% and and with a significance level (alpha) of 0.05 that there is no more than a 30% difference in cure rates between the group receiving the standard five day course of therapy (where based on our previous study we expect the rate of clinical cure to be 95%) and the two groups receiving the shorter courses of therapy. We estimate that we will have to initially enroll approximately 160 patients in order to achieve the number of patients we require for analysis. This allows for patients who do not have *Shigella* isolated from a culture of their admission rectal swab or stool

sample, as well as patients who withdraw from the study prior to its completion and who will therefore not be considered in the main analysis.

6. Outcomes evaluated

We will evaluate both the clinical and bacteriologic response to therapy. Clinical response be be judged on the basis of the patients last day of hospitalization, and will be graded as follows:

Resolution of illness:
1. No watery stools.
2. $\leq$ 3 stools in total for the 24 h period.
3. Afebrile ($<37.8^{o}C$)

Marked improvement:
1. Up to 1 watery stool.
2. $<$ 6 stools in total for the 24 h period.
3. Afebrile ($<37.8^{o}C$)

Slight improvement:
1. Up to 3 watery stools.
2. $\leq$ 9 stools in total for the 24 h period.
3. Afebrile ($<37.8^{o}C$)

Failure: Presence of any of the following:
1. > 3 watery stools.
2. $\geq$ 10 stools in total for the 24 h period.
3. Febrile ($\leq 37.8^{o}C$)

A patient will be considered bacteriologically cured if *Shigella* cannot be isolated from a day 3 stool or rectal swab sample, and cannot be isolated from any subsequent stool or rectal swab sample.

We will also compare a number of other clinical outcomes, including stool frequency on each of the six study days, the presence of physical

signs or symptoms such as abdominal pain or tenderness, and the number of WBC's on stool microscopic examination.

Only patients who complete all five days of therapy will be eligible to be included in the main analysis.

7. Handling of treatment failures

If at the end of treatment a patient is judged to be a treatment failure (by either clinical or bacteriologic criteria) they will receive a full five day course of ciprofloxacin, 500 mg q 12 hours for 10 doses.

8. Statistical methods

Differences between means will be tested using a one-way analysis of variance. Multiple comparisons of individual group means will be done using the multiple t-test, and will be carried out only if the overall F test is significant. For data that is not normally distributed, data will first be normalized by determining the normal log. Proportions will be tested using the chi-square test for m by 2 tables with partitioning of the degrees of freedom, and testing of multiple groups will be carried out only if the overall chi-square result is significant. If the expected cell size is less than five in any of the proportions being compared, Fisher's exact test will be used instead of the chi-square test. Statpac software (Walonick Associates, Minneapolis, Minnesota) will be used for the entry and sorting of data, and for statistical analysis.

9. Laboratory methods

Stool and rectal swab samples will be cultured by the routine methods now in use in our laboratories. Determination of drug levels will be done by a bio-assay in the laboratory of Dr. Michael Barza, of the Tufts University School of Medicine. Antibodies to Shiga toxin will done by an ELISA method using purified A (or B) sub-unit as the

capture antibody. In a selected number of these samples the ELISA method will be compared with a cytotoxicity neutralization assay.

10. Blood drawing

For this study we will withdraw a total of 26.6 ml of whole blood over a 16 day period in a total of 5 venipunctures and 8 blood draws (the multiple drug determinations on day 2 will be done through an indwelling venous catheter, which reduces the need for separate venipunctures for three of the blood draws).

11. Reimbursement of lost wages.

We believe that most patients, provided adequate therapy, will be able to return to work after their second day of treatment. Therefore patients will lose on average 5 days of work (4 days in hospital and 1 day for the follow-up visit). We will provide 150 taka/per day renumeration for the work days lost, as well as 150 taka transport costs for the return visit.

D. SIGNIFICANCE

If it is found that a short course ciprofloxacin therapy is as effective as standard five day, 10 dose therapy, then treatment can be greatly simplified, cost reduced, and presumably compliance increased. Determination of whether or not the anti-toxin antibody response is primarily directed against the A or B sub-unit of toxin will provide important information about the relative importance of the two different sub-units in initiating immunity to toxin, information that will be of use in developing a *Shigella* vaccine if it is found that toxin plays either an important role in pathogenesis (likely, at least in the case of HUS) or induces protective immunity. If the ZSR or the CRP appear

to correlate well with other signs and symptoms of disease, or with other laboratory measures of the intensity of colitis, such as ZSR and CRP, then they might be useful, simple measures for following the progress of disease.

E. COLLABORATIVE ARRANGEMENTS

The determination of the drug levels in serum and stool will be done in the laboratory of Dr. Michael Barza, Professor of Medicine, New England Medical Center, Tufts University School of Medicine.

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G. ABSTRACT SUMMARY

1. Patients enrolled in the study will be adults with clinical evidence of dysentery, and we will enroll only patients who are capable of giving voluntary informed consent. Children will not be enrolled in this study.

2. Our hypothesis is that short course therapy is as effective as standard five day therapy in the treatment of this infection. It is possible that short course therapy is less effective, and that patients receiving the shorter duration of therapy will have symptoms of dysentery for a longer period than patients who receive a 5 day course. Given the previous success of short course therapy with drugs that are less potent than ciprofloxacin, we think the latter possibility (that patients receiving a short course of therapy will have symptoms for a longer duration) unlikely.

3. Any patient who is judged to have failed treatment at the end of the five study days will be treated with a full five day course of medication. Thus, if required, all patients will receive known, effective therapy.

4. All data will be recorded and computerized using an anonymous study number.

5. Signed informed consent will be obtained from all patients prior to their entry into the study.

6. A medical history will be taken on admission to the study and daily. The interview will be done at the bedside, and will include only information that is routinely obtained as part of a medical history. The initial interview will last 15 to 20 minutes, and subsequent interviews approximately 10 minutes.

7. Patients will benefit because they will receive attentive care for their shigellosis. The major benefit however will accrue to society. If short course therapy is found effective, then this will have major public health implications for the treatment of dysentery in this country.

8. This study requires the use of Treatment Centre records, as well as the use of blood and stool samples.

Short course ciprofloxacin study

SECTION III - DETAILED BUDGET

A. Personnel:		% Period	Amount($)	Total ($)
Dr. M. Bennish	Pr.Investigator			
Dr. M.A. Salam	"	20% (2-yr)	5,612	
To be named	Medical Officer	100% "	16,800	
" "	Data Entry Tech	100% "	10,800	
" "	Laboratory Tech	100% "	19,218	52,430

B. Lab. test costs

Test	No. of test	Rate/test	
1a.stool M/E	120 x 3	1.65	594
1b.stool M/E	40 x 1	1.65	66
2a.blood for Hct%, T/C,D/C,retic, platelet, rbc morphology	120 x 1	6.93	832
2b.Do	40 x 1	6.93	278
2c.Do	30 x 1	6.93	208
3a.serum bilir.SGPT	120 x 1	6.6	792
3b.Do	40 x 1	6.6	264
3c.Do	30 x 1	6.6	198
4a.serum electrolyte, total protein, creatinine	120 x 1	7.0	840
4b.Do	40 x 1	7.0	280
4c.Do	30 x 1	7.0	210
5a.blood culture	120 x 1	8.0	960
5b.Do	40 x 1	8.0	320
5c.Do	10 x 1	8.0	80
6a.stool c/s for Shigella and Campylobacter	120 x 1	14.47	1,737
6b.Do	40 x 1	14.47	579
6c/Do	30 x 1	14.47	435
7a.rectal swab & stool culture for Shigella, and Salmonella	120 x 16	5.07	8,735
7b.Do	40 x 6	5.07	1,217
8a.determination of MIC	120 x 2	8.67	2,081

9. Antibiotic sensitivity	120 x 2	4.58	1,100	
10. Culture and complete biochemical iden.of non-cholera Vibrio	25	7.63	191	
11a.urine analysis	120 x 1	4.40	528	
11b. Do	40 x 1	4.40	176	
11c. Do	30 x	4.40	132	
12a.blood for C-reactive protein	120 x 3	2.00	720	
12b. " " "	40 x 1	2.00	80	24,633

C. Patient hospitalization -

1a.6 days/patient for 120 patients @ 30 US$/day (with the provision for 10% increment)	21,600	
1b.3 days/patient for 40 culture negative cases	3,600	25,200

D. Reimbursement of wage loss

For 120 study patients @ 3 $/day for 5 days/patient	3,000

E. Follow-up transport for 120 patients — 600

F. Supplies and material

1a. Study drugs (To be supplied free of cost)		
1b. needles, syringes, vials for sample collection, tape etc.	2,000	
1c. non-stock supplies.	2,000	4,000

G. ICDDR,B Transport	500
H. Transportation of things	1,500
I. Printing & reproduction	1,000
J. Telex, mimeograph	1,000
K. Capital expenditure	
1a. table top digital balance one	1,500
1b. File storage facility (cabinet)	200
Grand Total :	118,080

Budget Summary

		Amount (US$)
1.	Personnel services:	52,400
2.	Supplies and materials	31,180
3.	Equipment	1,700
4.	Patient hospitalization	28,800
5.	Outpatient care	-
6.	ICDDR,B transport	500
7.	Travel & transportation of persons	-
8.	Transportation of things	1,500
9.	Rent, Communication & utilities	1,000
10.	Information service	-
11.	Printing and reproduction	1,000
12.	Other contractual services	-
13.	Construction, renovation and alteration	-
	Total cost exclusive of support cost :	118,080

H. ENGLISH CONSENT FORM

You are suffering from blood dysentery which is probably due to infection with a germ named *Shigella*. The current therapy for this illness requires five days of therapy. We are trying to find out whether a shorter course of therapy, either for one, two or three days, is just as effective as the five day course. The drug that we will be using, ciprofloxacin, is known to be very effective in the treatment of this infection when given for five days, and we have good reason to believe that it will also be effective when given for shorter periods of time. Such a shorter course of therapy would be much more convenient, as well as less expensive. If you agree to participate in this study you will have a 1 in 4 chance of receiving the one day course of therapy, the two day course of therapy, the three day course of therapy, or the standard five day course of therapy. However neither you, nor we, will know which course you received until the study is completed. If you agree to participate in the study you will be required to:

1. Stay in the Treatment Centre for six days, and to come back for a return visit 10 days after discharge. Because we anticipate that you will be feeling substantially better after your second day of treatment, and you will therefore miss five days of work that you would otherwise not miss, we will reimburse you 150 taka/day for the five days that you miss, as well as 50 taka to cover the transportation costs of your return visit.

2. On admission to the study, and on each of the subsequent five days, we will take a detailed history of your symptoms, examine you thoroughly, and record the findings.

3. During the study (including the follow-up visit) we will withdraw 24.5 ml (about 5 teaspoons) of blood from you. This total amount will not be obtained at once, but rather in smaller amounts on 8 separate occasions. We will obtain this blood to ensure that you have no complications, as well as to determine your response to the infection, and the amount of the drug that is getting into your bloodstream. The amount of blood that we are obtaining will not in any way affect your health, and you will feel only a very slight pain, from the stick of a very small needle, at the time that we are withdrawing the blood.

4. On each of the study days we will insert a small cotton tipped swab into your rectum to obtain a sample which will let us know if you still

are infected with the germ that was causing your dysentery. This is not painful - on occasion it might cause you slight, momentary, discomfort.

5. We will also obtain a stool sample from you during every day that you are in the study for the same purpose - to tell whether you are still infected with the germ that is causing your dysentery.

6. During the period of the study your body temperature, pulse rate, and rate of respiration will be measured and recorded every four hours., and your blood pressure recorded at least twice daily. We will also ask you every day a number of questions about how you are feeling - questions such as whether you have a headache, stomach pain, and trouble breathing.

7. You are the one to decide if you should join in this study. If you do not participate in the study you will receive the usual and standard treatment that is offered by this Treatment Centre.

8. If at any time you wish to withdraw from the study you may so request.

9. All information gathered during this study will be kept private and confidential.

10. If you wish to know the results of any of the tests done during the study, or the overall study results, those will be provided to you upon your request, and as they become available.

If you agree to participate in the study, please put your signature or thumb impression below:

_____ _____

Signature of the Investigator Signature of the Patient

Date_____ Date_____