CHAPTER 1

INTRODUCTION

Background and rationale

Shigellosis or bacillary dysentery is an acute bacterial disease of the intestinal tract[1], one of the important infectious diseases in Thailand[21]. The cumulative reported cases of shigellosis in Thailand till December, 11th, of the year 1982, is 10,477 cases out of the total number of 291, 314 cases[2] of acute diarrhea.

The causative organisms of shigellosis is Shigella, a genus of bacteria in the family Enterobacteriaceae, the Gram-negative enteric bacilli group. Shigella, as well as many other Gram-negative bacteria, developed antimicrobial resistance mediated by resistance transfer factor (RTF)[22], the world wide spread of drug resistance.[22] The change in antimicrobial susceptibility of shigellae organisms was shown in table 1 and table 2. This is the problem we are facing, though it is not very serious now, it tends to be more serious by the increasing percentage of resistance to some commonly used drugs (see table 1,2). So, it needs some more attention to find another better treatment, especially, another effective antimicrobial agent for future use.

table 1[3,6]

Reports of antimicrobial susceptibility of Shigella showing the percentage of sensitive strains obtained from Ramathibodi hospital in 1973 - 1982

Year	1973	1974	1975	1978		1979		1981		1982	
organism / antimicrobial agents	Shigella spp.	Shigella spp.	Shigella spp.	S.flexneri	S.sonnei	S.flexneri	S.sonnei	S.flexneri	S.sonnei	S.flexneri	S.sonnei
ampicillin	87	85	67	28	60	13	76	12	43	13	3
carbenicillin	93	81	85	27	74	25	87	-	92	-	-
cephalothin	88	87	82	97	79	98	100	-	100	-	-
chloramphenicol	19	13	2	21	3	31	9	27	4	5	3
colistin	92	90	85	-	-	-	-	-	-	-	-
gentamicin	81	90	50	97	96	94	100	-	100	-	-
neomycin	72	78	20	-	-	-	-	-	-	-	-
nalidixic acid	-	93	-	-	-	-	-	-	100	-	-
co-trimoxazole	49	82	39	80	100	70	83	75	90	67	96
tetracycline	35	29	5	6	15	6	25	-	20	7	-

table 2[4,5]

Reports of Antimicrobial susceptibility of Shigella showing the percentage of sensitive strains obtained from Siriraj hospital in 1978 - 1980

Year	1978 [4]	1979	1980 [5]	
organisms / Antimicrobial agents	Shigella spp.	S.flexneri	S.flexneri	S.sonnei
ampicillin	30.5	9	19	41
carbenicillin	66.7	14	19	74
cephalothin	95.5	99	100	100
chloramphenicol	10.9	2	4	4
colistin	-	100	100	100
gentamicin	57.7	97	85	100
neomycin	34.1	87	63	81
nalidixic acid	98.7	100	100	100
co-trimoxazole	83.1	84	100	85
tetracycline	58.2	2	0	0

Besides these datae, there are many reports about Shigella's drug resistance[7,8] from other parts of the world.

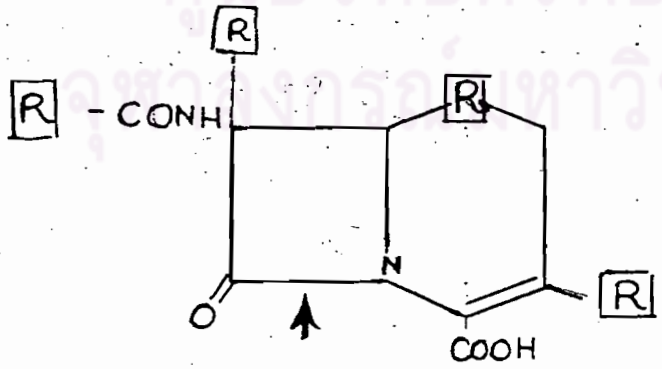
Because there has been increased resistance of Gram-negative

bacteria to existing compounds[10,11], and an increased awareness of the toxic potential of other anti-microbial agents, particularly the amino-glycosides[12], the proliferation of new antimicrobial compounds, especially the beta-lactam antibiotics have contributed:

The advances in chemistry have made possible the production of new cephalosporin or oxacephem[12] compounds with extraordinary in-vitro activity.

Cephalosporins consist of a dihydrothiazolidine ring fused with a four-membered beta-lactam ring. All of the cephalosporins posseses a sulfur atom at position 1 of the dihydrothiazolidine ring (see figure1) with the exception of moxalactam which has an oxygen atom. Substitutions at position 3 of the ring usually affect pharmacologic properties to a greater degree than microbiologic activity[12]. Substitution on the beta-lactam ring or near by affect stability against beta-lactamases, the enzymes that destroy these compounds, and further changes in the acyl side chain can alter both antibacterial and pharmacologic properties.[12]

figure 1[12] Basic cephem nucleus with R at positions in which major modifications are possible. Arrow indicates sites of beta-lactamase attack.



There are three aspects to the activity of penicillins and cephalosporins[12]; first, entry of the molecules through the outer bacterial cell wall, second, stability to beta-lactamases, and the third one, affinity for the enzymes involved in bacterial cell wall synthesis, the penicillin binding proteins (PBPs) which are located beneath the cell wall but outside the bacterial cytoplasmic membrane, in the periplasmic space[12]. They are the sites at which cephalosporin binds and inhibit cell wall production[12].

Gram negative bacteria, all have a complex outer cell wall and all contain beta-lactamases that can be plasmid mediated[12] (extrachromosomal DNA), or of chromosomal origin and inducible[12] which are contained in the periplasmic space (which differs from the Gram-positive bacteria which excreted them as the exoenzymes.) Finally, Gram-negative bacteria contain PBPs which bind with the beta-lactam antibiotics preferentially and may cause lysis, produce round forms or produce long filaments.

Classifications of cephalosporins

Cephalosporins are classified into 3 generations[12] as shown in table 3.

Table 3 Classification of cephalosporins

First generation	Second generation	Third generation
cephalothin	cefoxitin	cefotaxime
cephapirin	cefamandole	ceftizoxime
cephaloridine	cefuroxime	ceftriaxone
cephalexin	cefotiam	cefmenoxime
cephradine	cefmetazole	ceftazidime
cefaclor		cefoperazone
cefadroxil		moxalactam
cefonacid		cefsulodin
ceforanide		

Ceftriaxone is the new third generation parenteral cephalosporin with a long biological half life. It is very active against most Gram-positive and Gram-negative organisms (see page 15 for its antibacterial activity). Ceftriaxone has been raised for this research by the following concern and properties : -

1. It's high degree of stability to various types of beta-lactamases of many Gram-negative bacteria[14] (see table 14 p. 27)

2. Ceftriaxone is a long acting, broad spectrum antibiotic with an exceptional long biological half-life of 7.7 - 8.8 hours[13] while the other cephalosporins have relative shorter half life (between 0.5 and 2.7 hours).[15] So, single dose parenteral therapy should be considered to reduce the costs, and time of care to the patients.

3. From many reports (see pages 24-28), the in vitro activity of ceftriaxone to Shigella is excellent with the very low MIC

(between 0.012 - 0.2 µg/ml),[23,24,25] It's challenging to treat shigellosis, an invasive bacterial infection with ceftriaxone.

All of these mentioned data had motivated the researcher to handle the study of "Laboratory and Clinical Evaluation of Ceftriaxone to Shigellosis".

Objective

The purposes of this research are to determine the followings:-

1. to study the in vitro efficacy of ceftriaxone to shigellae of various groups and types.

2. to study the clinical and bacteriological efficacy of ceftriaxone against shigellosis in children with single-dose therapy.

3. to determine the serum concentrations of the drug at 1, 8 and 24 hours after administration.

Materials and Methods

This thesis is divided into two parts, the laboratory study and the clinical study.

1. The laboratory study

This part is the laboratory investigation of the following :-

1.1 The determination of antimicrobial susceptibility of Shigella of various groups and types to ceftriaxone. The other antimicrobial agents including ampicillin, cephalothin, chloramphenicol, TMP/SMX (co-trimoxazole) and tetracycline were tested against the microorganisms with the disc diffusion method[16,17]

1.2 Determination of Minimal Inhibitory Concentration (MIC)

of ceftriaxone to Shigella.

The agar dilution method[18] was used with a hand-held multipoint inoculum-replicating apparatus.[18]

The shigellae were isolates from the patients' rectal swabs or stool cultures from various parts of Thailand.There are 77 to 103 shigellae isolates tested. This part of study will provide the exact information of ceftriaxone susceptibility of Shigella in Thailand.

2. Clinical study

This part of the thesis is concerning the efficacy of ceftriaxone in treatment to the patients both clinically and bacteriologically and determinations of drug sera levels were included.

The drug was administered as a single dose of 50 mg per kilogram body weight intravenously or intramuscularly to the patients suffered with shigellosis. At least 20 children of age not more than 12 years were studied for 5 days or until the symptoms had been releived. The patients did not receive any other antimicrobial agents in the course of study. All patients were admitted, and most of them were in Bamrasnaradura Infectious Hospital, Nondhaburi.

2.1 Study for clinical efficacy of ceftriaxone

The following symptoms were observed daily for their improvements after drug administration :-

2.1.1 Fever

2.1.2 stool characteristics

2.1.3 the frequency of defecations

2.1.4 other gastro-intestinal symptoms

2.1.5 dehydration

2.2 Study for bacteriological efficacy of ceftriaxone in treatment of shigellosis

The rectal swabs or stool cultures of the patients were checked for the presence of shigella daily after ceftriaxone administration.

This part was done by the clinical microbiology laboratories, Department of Pathology, Bamrasnaradura Infectious Hospital. This study would give the data of bacteriological efficacy of ceftriaxone

These two parts (2.1 and 2.2) were studied and observed for 5 days or until the day of discharge.

2.3 Determination of serum levels of ceftriaxone after administration

The sera were obtained from the blood drawn at 1, 8 and 24 hours after injection of ceftriaxone. They were frozen at -20° C until the time of assay. The microbiological assay of antibiotics[19] by the plate method[19] was used. The details of materials and methods were described in chapter 3 page 61

The sera levels of ceftriaxone were done to give the exact informations for Thai children in this study. These data may be used to describe the results of ceftriaxone treatment.

Significance of the study

1. If a single dose of ceftriaxone is found to be effective in treatment of shigellosis, the advantages of this are :-

1.1 to reduce the number of drug administrations from multidose treatment for a long period (more than 3 days, see pages 51 chapter 2) of other microbial agents to only one dose treatment, which will be convenient and more economical.

1.2 to reduce the admission time. This because the clinical symptoms especially fever were observed to be improved in a very short time in the pilot project.

1.3 it will mean medical progress in finding another new antimicrobial agent for present or future use in treatments of shigellosis, the disease with a tendency of higher drug resistance to the causative organisms (see table 1,2 p. 2,3)

2. The in-vitro study of ceftriaxone efficacy to Shigella will give the exact information of drug susceptibility for Thailand's shigellae which were isolated from Thai patients, which were the true pathogenic strains.

3. The determination of serum levels of ceftriaxone in Thai children will give the exact information for Thai children, the difference in tribes from the other reports of other part of the world[20]. This is the information to be applied in the treatment of other diseases with ceftriaxone.