Azithromycin: Is it a favorable alternative therapeutic option against salmonella species ?

Dr.Bassim Irheim Mohammed Al Sheibani MBChB, MSc.** **Dr.Manal Mohammad Kadhim MBChB, MSc Dr.Sameer Hasen Abood Al Rekabi MBChB,D.M, MSc*

Abstract

Background: For decades chloramphenicol has been highly effective against *S. typhi* and *S.paratyphyi* . However the known hematological side effects of chloramphenicol and the widespread emergence of multiple drug resistance (MDR) to *S. typhi* has necessitated the search for other therapeutic options .

Aim of study: To compare between azithromycin and chloramphenicol as antimicrobial drugs for isolated salmonella species

Materials and Methods: 65 isolates of *salmonella spp*. was collected by performing blood culture and stool culture for suspected patients with history of typhoid fever and diarrhea at Maternity and Child teaching Hospital in Diwaniyah city. For each isolates antimicrobial susceptibilities for azothromycin and chloramphenicol were determined by Disk diffusion methods.In addition, a broth dilution tests for azithromycin were performed by using serial dilution of concentrations ranging from 4- 32 mg\ liter to record the minimal inhibitory concentration (MIC) for azithromycin .

Results: The isolated salmonella species are high susceptible of to azithromycin in comparison to chloramphenicol with lower MIC.

Conclusion: though it needs further clinical studies, azithromycin seems to be a suitable therapeutic option for the treatment of typhoid fever in children as well as in adult.

Introduction

Salmonella species(spp.),a highly evolved gram-negative bacterial parasite that infects humans[(1)],and some times cause fatal infection in adults and children as a result of bacteremia and inflammatory destruction of the intestine and other organs ,it is endemic in most countries ,especially throughout Asia and Africa[(2)]. For decades chloramphenicol has been highly effective against *S. typhi* and *S.paratyphyi* ,and it often remains the antibiotic of choice for the treatment[(3,4)].However ,the widespread emergence of multiple drug resistance (MDR) to *S. typhi* has necessitated the search for therapeutic options. Fluoroquinolones have proven to be effective, however, to date, they are restricted from routine use in children , pregnancy and lactation[)]. The cephalosporins; cefotaxime ,ceftriaxone and cefixime are useful against MDR

typhoid fever , but the required intravenous route of administration render them impractical for some patients [5,6,7].

Azithromycin is the first of a new class of brood spectrum antibiotics called azalides . Azithromycin ,a15 –atome lactone macrolide ring compound, is derived from erythromycin by addition of methylated nitrogen into the lactone ring of erythromycin with better activity than erythromycin against gram-negative bacteria[8].Azithromycin penetrates into most tissues (except cerebrospinal fluid) and phagocytic cell extremely well, with tissue concentrations exceeding serum concentrations by 10-100 fold. The drug is slowly released from tissues (tissue half-life of 2-4 days) to produce elimination half-life approaching 3 days. These unique properties permit once-daily dosing and shortening of the duration of the treatment in many cases. Because it has a 15-member lactone ring, azithromycin does not inactivate cytochrom P-450 enzymes and therefore is free of drug interaction that occurs with macrolides (9).

Aim of the present in vitro study is to compare the antimicrobial activity of azithromycin versus chloramphenicol for isolated *salmonella spp*. through disk diffusion test and minimal inhibitory concentration (MIC).

Materials and methods

Over six month period, 65 isolates of *salmonella spp*. Was collected by performation blood culture and stool culture for suspected patients with history of typhoid fever and diarrhea, at Maternity and Child Teaching Hospital . Isolates confirmed by biochemical reactions and agglutination antisera as *S.typhi* and *S. paratyphyi* . For each isolates antimicrobial susceptibilities were determined by Disk diffusion methods according to the procedure performed by NCCLS ,National committee for clinical laboratory standards, 2000 by preparing a suspension of salmonella species colonial growth from an over night culture on XLD(xylose lysine deoxycholate)agar in tube with 2ml of muller- Hinton broth and adjust to turbidity equivalent to McFarland standard equal to 0.5 ($1.5x\ 10^8$ CFU\ml),and we inoculated the muller –Hinton plate evenly by streaking it with prepared suspension across its surface, antibiotic disks of azothromycin and chloramphenicol were applied within 3-5 minutes after inoculation, thereafter incubated at 35-36$C^o$for 24 hours then we measure the inhibitor zone(10) .
Species were considered as susceptible when zone diameters for azithromycin disks containing 15ug were equal or more than 13 mm, and for chloramphenicol disk containing 30 ug were equal or more than 12mm, Finally a broth dilution tests for azithromycin were performed by using serial dilution of concentrations ranging from 4- 32 mg\ liter , the lowest

antimicrobial concentration that completely inhibits bacterial growth is recorded as the minimal inhibitory concentration (MIC) for azithromycin.

Results

Sixty- Five isolates of *salmonella* species confirmed by biochemical reactions and agglutination antiseria as *S.typhi* in 45 case and *S.paratyphi* in 20 case ,and by using zone diameter equal or more than 13 mm for azithromycin, susceptibility to azithromycin was reported in 51(78.4%) from 65 isolates , and by using zone diameter equal or more than 12mm for chloramenicol ,susceptibility was reported in 50(76.9%) from 65 isolates table (2). Azithromycin MICs test were in range of 4-38 mg /l ,table(3). The isolates of *S .typhi* had lower MICs than isolates of *S. paratyphi* ,and most of *S. typhi* inhibited by azithromycin 4 mg/l , while most of *S. paratyphi* inhibited by azithromycin 16 mg|l .also there are 2 isolates of *S.typhi* was more resistance in repeated testing than other MIC equal or more than 32 mg /l.

Table (1): Characteristic of 65 cases with culture positive salmonella spp.

Parameter	Number(NO.)
Age(years)	
(2-10)	65
Sex	
Mal	43
Female	22
Patients with positive :	
- blood culure with S. typhi	20
- Stool culture with S.typhi	25
-Blood culture with S. paratyphi	8
-Stool culture with S. paratyphi	12
Total	65

Table(2):In vitro susceptibilities of 65 isolate of salmonella spp. for azithromycine versus chloramphenicol.

Disk diffusion	NO .of isolates(%)
Salmonella typhi (n=45)	
-Azithromycin susceptible or intermediate	33
-Azithromycine resistance.	12
-Chlormphenichol susceptible or ntermediate.	30
-Chloramphenicole resistance.	15
Salmonella paratyphi(n=20)	18
-Azithromycin susceptibile or intermediate.	2
-Azithromycin resistence.	20
-Chloramphenicol susceptible or intermediate.	0
-Chloramphenicol resistence.	**5**
Total Salmonella spp. Sensitive to azithromycin	(78.4%)
Total Salmonella spp. Sensitive to chloramphenicol	50(76.9%)
Total NO. of isolates	**65**

Table(3): MICs in mg/L of azithromucin for 65 isolate of salmonella spp..

Concentration for S. typhi	NO. of isolates
-4	**30**
-8	**12**
-16	**1**
->32	**2**
Concentration for S.paratyphi	**NO. of isolates**
-4	**0**
-8	**3**
-16	**17**
>32	**0**

Discussion

Chloramphenicol has been the drug of choice for typhoid fever for more many decades in the regions of the world where salmonella typhi remains susceptible to the drug .However the known hematological side effects of chloramphenicol and the multiple drug resistance to ampicilline, chloramphenicol, trimethoprim-sulphamethaxazol in Salmonella typhi that emerged in many countries of Asia and Afarica limit the usefulness of these drugs(11). Fluoroquinolones , ciprofloxacin and ofloxacin have proven to be effective, however, to date, they are generally not approved for use in children , pregnancy and lactation because of the potential for these drugs to damage cartilage in growing bones[(12)]. The cephalosporins; cefotaxime ,ceftriaxone and cefixime are useful against MDR typhoid fever , but the required intravenous route of administration render them impractical for some patients [(7)].The availability of pediatric suspension of azithromycin, the drug ability to achieve intracellular concentration and the long half-life are all encouraging features to use azithromycin in salmonella typhi diseases.

In the current study we observed high percentage of resistance to chloramphenicol .These results was expected because of the high prevalence of multi-drug resistance to chloramphenicol ,ampicillin, co-trimoxazole and ciprofloxacin [(5)].

The results of the present in vitro study indicated that azithromycin active against strains of *S.typhi* and *S.para typhi* ,regardless of whether they are multiple–drug resistance or susceptible to other drugs such as ampicillin and chloramphenicol. These findings are similar to that by Butler T et al 1999 (13).

The present study showed that out of 45 S.typhi isolates , 43 are susceptible to azithromycin .Resistance was not expected because this drug has not been used extensively in our country. When 45 isolates of *S.typhi* were tested for azithromycin MICs, all isolates except 2 susceptible ,with MICs of 4-8mg|l ,this difference between results of susceptibility testing by disk zone diameter and MICs suggests that there was less azithromycin resistance in our isolates of *S.typhi* than was reported initially from results of disk diffusion. Our results were agree with recent study conducted by Egyptian Ministry Health which proved that oral azithromycin administered once daily appears to be effective for the treatment of uncomplicated typhoid fever in children[(9)].

The availability of pediatric suspension of azithromycin, the drug ability to achieve intracellular concentration , the long half-life and large safety margin are all encouraging features to use azithromycin in salmonella typhi diseases in pediatric age group. The place of azithromycin in the treatment of typhoid fever needs to be defined by further clinical studies with adults and children.

References

1-Parker,M.T.1983.Principles of bacteriology, virology,and immunology. 17th ed.Eduard Arnold,London,England,P.332-355.

2-Azad,A.K.and T.Butler.1997.Comparation of clinical features and pathologic finding in fatal cases of typhoid fever during initial and later stages of disease .Am.J.Trop.Med.Hyg.56:490-493.

3-Acharya G. and ButlerT,1995. Treatment of typhoid fever: randomized trial of a three-day course of ceftriaxon versus afourteen-day course of chloramphenicol .Am J Trop Med Hyg ;52:162

4. Acharya G, Butler T, Ho M, et al. Treatment of typhoid fever: randomized trial of a three-day course of ceftriaxone versus a fourteen-day course of chloramphenicol. Am J Trop Med Hyg 1995; 52:162–5.

5. Islam A, Butler T, Kabir I, Alam NH. Treatment of typhoid fever with ceftriaxone for 5 days or chloramphenicol for 14 days: a randomized clinical trial. Antimicrob Agents Chemother 1993; 37:1572–5.

6. Wain J, Hoa NTT, Chinh NT, et al. Quinolone-resistant *Salmonella typhi* in Vietnam: molecular basis of resistance and clinical response to treatment.Clin Infect Dis 1997;25:1404–10.

7. Vinh H, Wain J, Vo TN, et al. Two or three days of ofloxacin treatment for uncomplicated multidrug-resistant typhoid fever in children. Antimicrob Agents Chemother 1996; 40:958–61.

8-Neu.H.C (1991). Clinical microbiology of azithromycin.American journal of medicine 91.Suppl 3A, 12S-8S.

9-Basic and clinical pharmacology 2006 by Katzing

10- NCCLS,(2000).Performance standards for antimicrobial Disk Susceptibility Tests, Approved Standard ,seventh Edition, Wayne,Pennsylvania.

11. Mirza, S. H., N. J. Beeching, and C. A. Hart. 1996. Multi-drug resistant typhoid fever: a global problem. J. Med. Microbiol. 44:317–319.

12.. Cao XT, Kneen R, Nguyen TA, Truong DL, White NJ, Parry CM. A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children. Dong Nai Pediatric Center Typhoid Study Group. Pediatr Infect Dis J 1999; 18:245–8.

13-Butler T, Sridhar CB, Daga MK, et al. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicentre trial in India. J Antimicrob Chemother 1999;44:243-50.