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Comparison of the Antibacterial Efficacies of Co-trimazine and Co-trimoxazole Against *Listeria monocytogenes* in Cyclosporin A Treated Mice

Abbas Rezaee and Qurban Behzadiannejad

Listeria monocytogenes is a facultative, intracellular, gram positive bacterium that is responsible for sever infections, including prenatal infections, septicemia and meningoencephalitis in humans and a wide variety of animal species. In this study, efficacies of co-trimazine in an immunosuppressed mouse model of listeriosis was compared with co-trimoxazole. After treatment with co-trimazine (400 mg kg^{-1} of sulphadiazine and 80 mg kg^{-1} of trimethoprim) every 12 h for 3 days, *Listeria monocytogenes* could not recovered from the livers of the mice. In contrast, after treatment with co-trimoxazole (400 mg kg^{-1} sulphamethoxazole and 80 mg of trimethoprim) every 12 h for 3 days, a mean of 3×10^3 colony-forming unit (CFU) was recovered from the livers of mice. Results showed that antibacterial efficacy of co-trimazine was more than co-trimoxazole in experimental infections with *Listeria monocytogenes*.

Key words: *Listeria monocytogenes,* co-trimazine, co-trimoxazole, antibacterial efficacies

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For further information about this article or if you need reprints, please contact:

Abbas Rezaee
Department of Microbiology,
Faculty of Medical Science,
Tarbiat Modarres University,
Tehran, Iran

E-mail: abbasrezaee@yahoo.com
rezaee@mail,modares.ac.ir
Fax: +98 21 8013030

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Department of Microbiology, Faculty of Medical Science,
Tarbiat Modarres University, Tehran, Iran

Introduction

Listeria monocytogenes has been recognized as a human pathogen for several years (Gellin and Broom, 1989). This bacteria cause illness mainly in pregnant women, newborns, elderly persons and immunocompromised persons (Cossart and Mengaud, 1990; Pong and Bradley, 1999; Smith, 1999).

Chemotherapy is not very effective in systemic listeriosis, although *Listeria monocytogenes* is susceptible to most of the antibiotics used for therapy (Hof *et al.*, 1997; Michelet *et al.*, 1994). Resistant strains are rare. In contrast to the usual *in vitro* susceptibility of this pathogen to most antibiotics, listeriosis induces a high rate of mortality, ranging from 21 to 44.4% (Ogtrop *et al.*, 1992). There are several possible reasons for this:

(a) The frequent occurrence of listeriosis in immunocompromised patients, including neonates, patients with underlying diseases
(b) The intracellular site of bacterial replication, in which *Listeria monocytogenes* is capable of multiplying inside the cytoplasms of many cells, including macrophages, epithelial cells, fibroblasts and hepatocytes, thus impeding the bactericidal activities of antibiotics *in vivo*
(c) The high incidence of meningoencephalitis, producing sever lesions of the central nervous system, where antibiotic penetration might be restricted (Michelet *et al.*, 1994; Ogtrop *et al.*, 1992; Pong and Baradley, 1999).

The recommended treatment for listeriosis remains amoxicillin alone or combined with gentamicin, regardless of their apparently weak intracellular diffusion and the absence of synergy between these antibiotics, which has been observed during the course of experimental infections in animals (Hof *et al.*, 1997; Nichterlein and Hof, 1991). The combination of trimethoprim with a sulfonamide such as sulphamethoxazole, as in co-trimoxazole, has been regarded as a drug of second choice for the treatment of listeriosis (Hof *et al.*, 1997 and Michelet *et al.*, 1994). In view of the high rate of clinical failures observed with the proposed treatment, alternative treatments have been based on the high degree of intracellular penetration of certain antibiotics (Reeves, 1982). The sporadic nature of listeriosis explains the absence of any controlled clinical trials establishing the superiority of one particular therapeutic protocol (Robibins *et al.*, 1999).

The aim of the research work was to comparison of the antibacterial efficacies of co-trimazine and co-trimoxazole against *Listeria monocytogenes* in liver of mice immunocompromised by treatment with CS-A .

Materials and Methods

Mice: Seven to 8 week-old female Balb/c mice were purchased from Razi Institute, Tehran, Iran. For experiments, the animals were transferred to Tarbiat Modarres University.

Antimicrobial activity: For testing the activity, trimethoprim, sulphadiazine and sulphamethoxazole were all obtained from Sigma Chemical Co. in the form of powders suitable for susceptibility testing.

Bacteria: *Listeria monocytogenes*1/2a (NCTC 7973) strain was used in the study. It was grown in brain heart infusion broth (BHI) and was stored at -70°C in the form of 1 ml aliquot containing 1.5 x 10^3 bacteria per ml in the growth phase. In each experiment, an aliquot was thawed, inoculated into 200 ml of BHI broth and incubated at 37°C for 15 to 16 h. The organisms were collected by centrifugation and resuspended in BHI broth. The concentration of the cell suspension was adjusted with 0.5 Mcfarland turbidity standard then diluted.

Minimum inhibitory concentration (MICs): The MICs were determined by the agar dilution method on iso-sensitest agar (oxoid). The MICs of trimethoprim, sulphadiazine, sulphamethoxazole, co-trimazine (sulphadiazine and trimethoprim) and co-trimoxazole (sulphamethoxazole and trimethoprim) were determined (Michelet *et al.*, 1994). Fractional inhibitory concentration index (FIC Index) was calculated as described by Sabath (Richars *et al.*, 1995).

Treatment with CS-A: CS-A was obtained by Sandoz, Switzerland. It was dissolved in normal saline. Seventy five mg of the drug was injected intra-peritoneally 2 day before injection of *Listeria monocytogenes*. Control mice were injected with saline normal. Mice were treated once daily with 75 mg of CS-A intra-peritoneally.

Experimental infection: Infections were induced by injection of 5 x 10^3 CFU of *Listeria monocytogenes* into tail vein of immunocompromised mice. The numbers of viable *Listeria monocytogenes* recovered from the liver were used as indices of the severity of infection. Mice were sacrificed at different intervals after inoculation (24, 48 and 72 h) and livers were removed and homogenized separately in 20 ml of physiological saline. Tenfold serial dilutions of homogenates in saline were prepared and 0.2 ml volumes of each dilution were spread on blood agar plates.

***In vivo* effect of co-trimazine and co-trimoxazole on the growth of *Listeria monocytogenes*:** The effect of antimicrobial treatment was assessed in CS-A treated mice. Two days after the first injection of CS-A, *Listeria monocytogenes* was injected i.v. twenty-four hours after the injection of *Listeria monocytogenes*, the antimicrobial treatment was started. The doses used for co-trimazine were sulphadiazine 400 mg per kg of body weight and 80 mg of trimethoprim per kg while for co-trimoxazole, 400 mg per Kg sulphamethoxazole and 80 mg kg^{-1} of trimethoprim simultaneously every 12 h for 3 days.

Statistical analysis: The results of assessment of the quantitative cultures are given as the mean log CFU per organ. One-way analysis of variance was used for statistical comparisons (Hugin *et al.*, 1986).

Results

The combination of trimethoprim-sulphadiazine (co-trimazine) and trimethoprim-sulphamethoxazole (co-trimoxazole) showed an enhanced activity by exhibiting a decreased FIC indices of 0.73 and 0.78 respectively (Table 1). The MIC of different drugs in combination and alone were ranged from 8 to 310 mg l^{-1}.

Table 1: MICs and FIC of trimethoprim, sulphadiazine and sulphamethoxazole alone or in combinations against *Listeria monocytogenes* 1/2a

Antibacterials	MIC (mg l^{-1})	FIC (index)
Trimethoprim	16	----
Sulphadiazine	180	----
Sulphamethoxazole	310	----
Co-trimazine	8	0.73
Co-trimoxazole	29	0.78

MIC: Minimum inhibitory concentration
FIC: Fractional inhibitory concentration

Table 2: Effect of CS-A on the outgrowth of *Listeria monocytogenes* in the liver of mice

Days after infection	CS-A treatment	No. of *Listeria monocytogenes* (log_{10}) per liver
1	-	3.6±0.3NS
	+	3.9±0.7
2	-	4.2±0.5*
	+	5.2±0.2
3	-	4.3±0.1*
	+	6.9±0.4

CS-A: Cyclosporin A *: $P<0.05$ NS: Non-significant
+: Experimental (treated) mice - : Control mice

Table 3: Outgrowth of *Listeria monocytogenes* in the liver of mice after administration of a single intraperitoneal dose of co-trimazine

Time (h)	Co-trimazine	No. of *Listeria monocytogenes* (log_{10}) per liver
24	-	3.9±0.7
	+	2.6±0.4
48	-	5.2±0.2
	+	3.8±0.6
72	-	6.9±0.4
	+	4.6±0.9

Table 4: Effect of Co-trimazine administered intra-peritoneally twice times daily on the number of CFU of *Listeria monocytogenes* in the liver of mice treated with CS-A

Time (h)	Co-trimazine	No. of Listeria monocytogenes (log_{10}) per liver
24	-	3.9±0.7NS
	+	2.9±0.6
48	-	5.2±0.2*
	+	2.8±0.3
72	-	6.9±0.4*
	+	2.3±0.9

Table 5: Effect of co-trimoxazole administered intraperitoneally twice times daily on the number of CFU of *Listeria monocytogenes* in the liver of mice treated with CS-A

Time (h)	Co-trimoxazole	No. of *Listeria monocytogenes* (log_{10}) per liver
24	-	3.9±0.7NS
	+	3.4±0.4
48	-	5.2±0.2*
	+	3.2±0.2
72	-	6.9±0.4*
	+	2.9±0.9

Results (Table 2) showed that after Ist day infection the no. *Listeria monocytogenes* was declined (3.9±0.7) while after 2nd (5.2±0.2) and 3rd day (6.9±0.4) of infection it was increased significantly ($P < 0.05$).

The mice were killed 24, 48 and 72 h after administration of the antimicrobial agent. Preliminary experiments showed that after administration of a single intraperitoneally dose of co-trimazine, the number of *Listeria monocytogenes* cells in the livers was initially declined (2.6±0.4) after 24 h but regrowth of *Listeria monocytogenes* was occurred (3.8±0.6 and 4.6±0.9) after 48 and 72 h of drug administration (Table 3). Treatment with antimicrobial agent twice a daily led to a much larger reduction in the number of *Listeria monocytogenes* in the livers of mice. Results showed that the number of *Listeria monocytogenes* was 2.9±0.7 after 24 h, while it was decreased significantly ($P < 0.05$) after 48 and 72 h (2.8±0.3 and 2.3±0.4) of treatment with co-trimazine (Table 4). The numbers of *Listeria monocytogenes* declined to below the detection limit (500 CFU per organ) at day 3. Treatment with co-trimoxazole (Table 5) also led to a significant ($P < 0.05$) decrease in the number of *Listeria monocytogenes* cells in the livers, but decrease was less than that after treatment with co-trimazine (Table 4).

Discussion

Testing of the effect of antibiotics against intracellular bacteria may be influenced by several factors, for example *in vitro* susceptibility, up take of the antibiotics by the cells and distribution of the antibiotics by the cells and distribution of the antibiotics within body (Nichterlein and Hof, 1991). The therapeutic efficacy of conventional treatment is still problem, at least in part because of the habitat of the organisms within host cells. Listeriosis is not a major problem in medicine, but the high mortality rate of overt listeriosis in very young, old and immunocompromised patients represents a challenge for medical microbiologists, clinicians, veterinarians and food microbiologists (Gellin and Broome, 1989). Though practically all strains of *Listeria monocytogenes* are susceptible *in vitro* to the most commonly used drugs, but therapeutic results are not always satisfactory. Thus, further efforts have been made to find an optimal drug or drug combinations to achieve an improved therapeutic index. Several studies have been published on the *in vitro* activity of antimicrobial agents against *Listeria monocytogenes* by testing different kinds of substances (Hof *et al*., 1997). Results from different studies indicate a good homogeneity among the *Listeria monocytogenes* with respect to the MICs. Results of the present study showed that *Listeria monocytogenes* was sensitive to co-trimazine and co-trimoxazole (Table 1). In practice, the choice of therapy is limited in spite of the large array of antibiotics activity *in vitro*. Ampicillin or amoxicillin, combined with an amino glycoside remains the primary choice. The amino glycoside should be added because it enhances the bactericidal action of penicillins. Co-trimoxazole has been proposed for use in patients, it is hypersensitive to β-lactam antibiotics and has also been recommended as a first-line treatment because of the good diffusion of sulphamethoxazole and trimethoprim into the meninges (Wormser *et al*., 1982).

Results of the present study (Table 4) showed that co-trimazine exhibit antibacterial activities against *Listeria* infection in mice which are immunocompromised by treatment with CS-A and found that antibacterial effect of co-trimazine was more than co-trimoxazole (Table 5). It may be due to that co-trimazine was more potent *in vitro* than co-trimoxazole against *Listeria monocytogenes* and that the *in vivo* concentration of co-trimazine was much higher than co-trimoxazole. It has been shown that co-trimazine penetrates easily into phagocytes (Richards *et al*., 1995). Since it has also been shown that *Listeria monocytogenes* migrates from macrophages into hepatocytes in mice (Portnoy *et al*., 1992), it might well be that co-trimazine effects this process more effectively than co-trimoxazole doses. Because co-trimazine is more effective in decrease of outgrowth of *Listeria monocytogenes* in liver (Table 3). In the most common clinical setting of meningitis, *listeriae* behave more as extracellular than intracellular pathogens, and therefor antibiotics that exert a good activity *in vitro* should be considered for the initial therapy of listeriosis. Since intrinsic resistance except to some antibiotics is uncommon and acquired resistance is extremely rare, many options are available. Unfortunately only few agents are rapidly bactericidal *in vivo* for *Listeria monocytogenes* (Hof *et al*., 1997; Michelet *et al*., 1994). Furthermore, because meningitis and encephalitis are prominent clinical manifestations of disease, drugs that easily cross the blood-csf barrier and the blood-brain barrier must be chosen (Altimira *et al*., 1999; Conlan and North, 1992). Whereas antibiotics may have a good chance to act on listeria in the more permeable inflamed meninges and the ventricles, the blood-brain barrier is more complex in normal situations and possibly more restrictive even in the case of inflammation.

In conclusion, co-trimazine was more potent *in vitro* than co-trimoxazole against the strain of *Listeria monocytogenes*

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