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Vancomycin versus teicoplanin in the therapy of experimental methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis

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Abstract

The aim of this study was to compare the antibacterial activity of teicoplanin and vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis using a rabbit meningitis model. The MRSA strain ATCC 43300 was used to infect the rabbits. The vancomycin group received 20 mg/kg vancomycin every 12 h (q12h), the teicoplanin group received 6 mg/kg teicoplanin q12h and the control group did not receive any treatment. Drug levels were measured using a bioassay technique. Bacterial counts in the treatment groups were significantly lower ($P < 0.05$) than those of the control group at 12 h and 24 h after treatment. When the treatment groups were compared, the bacterial counts after 12 h or 24 h of treatment were similar ($P > 0.05$). These data suggest that the antibacterial activity of vancomycin and teicoplanin are similar in experimental MRSA meningitis of rabbits.

Keywords: Pharmacokinetics; Meningitis; MRSA; Bioassay; Teicoplanin; Glycopeptides; *Staphylococcus aureus*

1. Introduction

Staphylococcus aureus is an emerging cause of bacterial meningitis [1] and is associated with a 27–36% mortality rate [2–4]. It is usually associated with neurosurgical interventions, staphylococcal bacteraemia or a parameningeal focus [5]. Methicillin-resistant *S. aureus* (MRSA) is a global problem [6–8] and has emerged as an important cause of hospital-acquired central nervous system infections [3–5]. Although the main therapeutic choice is vancomycin [6], there are several reported cases treated with intrathecal or intravenous teicoplanin [9–12].

To our knowledge, there is no human or animal study comparing teicoplanin and vancomycin in MRSA meningitis. In this study we compared the antibacterial activity of teicoplanin and vancomycin in the treatment of MRSA meningitis in an experimental rabbit meningitis model.

2. Materials and methods

2.1. Test organism

The inoculum was MRSA strain ATCC 43300. The minimum inhibitory concentration (MIC) of both teicoplanin and vancomycin was 1 mg/L (measured in duplicate using the Etest; AB BIODISK, Solna, Sweden).

2.2. In vivo studies

Male white New Zealand rabbits weighing 2–2.5 kg were anaesthetised by intramuscular ketamine (35 mg/kg) and

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xyszlazine (5 mg/kg) before each intraventricular intervention including induction of meningitis and cerebrospinal fluid (CSF) sampling [13]. The duration of anaesthesia was 10–15 min.

Meningitis was induced by direct inoculation of 0.3 mL physiological serum containing 10^7 colony-forming units (CFU)/mL MRSA into the cisterna magna of rabbits using a 22 G syringe (Hayat Ticaret, İstanbul, Turkey) [13].

After 16 h incubation, rabbits were separated into three groups: Group V, vancomycin; Group T, teicoplanin; and Group C, control. Group V received 20 mg/kg vancomycin (Lilly, Indianapolis, IN) every 12 h (q12h) (at 16 h and 28 h after the induction of meningitis); Group T received 6 mg/kg teicoplanin (Aventis-Pharma, West Malling, UK) q12h (at 16 h and 28 h after the induction of meningitis); and Group C did not receive any treatment. Drugs were infused as 10 mL solutions into the external vein of the ear of the rabbits over a 5-min period.

Meningitis criteria were as follows: fever (>40 °C); CSF pleocytosis of >1000 cells with >96% polymorphonuclear leukocytes; and a CSF bacterial count $>10^3$ CFU/mL [13].

CSF samples (0.1–0.25 mL) were obtained 28 h and 40 h after induction of meningitis by puncture of the cisterna magna using a 25 G needle (Hayat Ticaret) as used for lumbar puncture [13]. At 40 h, blood (5 mL) was sampled by cardiac puncture and serum was obtained via centrifugation. Animals were kept comfortably in their cages between interventions and they were permitted water and feed ad libitum. At the end of the study period (40 h), animals were humanely killed by intravenous infusion of high dose nembutal.

The bacterial count in CSF was measured by standard serial dilutions of 50 μL CSF in 0.9% NaCl and incorporation into sheep blood agar (Oxoid, Basingstoke, UK) pour plates [13]. The limit of detection of bacterial counts was 2×10^2 CFU/mL.

The evaluation of bacteriological response was defined using three categories: full response, sterilisation of CSF; partial response, any decrease in bacterial count; and bacteriological failure, a stable or increased bacterial count.

2.3. Antibiotic assay

Levels of teicoplanin and vancomycin were measured twice by a bioassay technique using *Bacillus subtilis* (ATCC 6633). Standards were prepared fresh on the day of use in pooled rabbit serum and a phosphate buffer solution containing 150 mmol/L NaCl and 80 mmol/L $CaCl_2$. Assay curves were produced using standard dilutions including 0.5, 1, 2, 4, 8, 16, 32 and 64 mg/L teicoplanin or vancomycin. A concentration of 20 mg/L drug (teicoplanin or vancomycin) including control rabbit sera was used for each test [14–16]. The assay had a good reproducibility (±10%). The sensitivity of the assay was 1 mg/L for both drugs.

2.4. Statistical analysis

Data were evaluated by SPSS 11.0 package program using Mann–Whitney *U*-test, Kruskal–Wallis test and Fisher's χ^2 test. A *P*-value less than 0.05 was considered significant.

2.5. Ethical issues

The study protocol was approved by the local ethical committee on animal studies (Approval No. 2003-50).

3. Results

At the beginning of the study, 45 animals were inoculated with MRSA, of which 39 were alive at the end of 16 h incubation time. These 39 animals were separated into three groups each consisting 13 animals.

At 16 h, all animals had developed meningitis and CSF bacterial counts were similar in all groups ($P > 0.05$) (Table 1). At 28 h (12 h after the end of the incubation time) or at 40 h (24 h after the end of the incubation time, and the end of the study) bacterial counts in Groups V and T were significantly lower ($P < 0.05$) compared with Group C (Table 1). There was no significant difference ($P > 0.05$) between treatment groups at either 28 h or 40 h (Table 1).

During the study, mortality among animals was similar in all three groups (Table 2). When Groups V and T were compared at 40 h, rates of partial bacteriological response (two in Group V, seven in Group T), full bacteriological response (two in Group V, one in Group T) and full or partial bacteriological response were similar ($P > 0.05$).

At 40 h, the serum drug levels were also similar (Group V, 7.9 ± 3.64 mg/L; Group T, 10.8 ± 5.6 mg/L; $P > 0.05$). The CSF drug level was higher than the lowest drug detection limit of the bioassay (1 mg/L) in only six rabbits: four rabbits in Group T (2.5, 3.2, 4.2 and 8 mg/L) and two rabbits in Group V (2 and 2.9 mg/L) ($P > 0.05$). The CSF:serum ratio ranged

Table 1
Results of bacteriological cerebral spinal fluid cultures

Treatment group	Bacterial count ($\log_{10}$ CFU/mL)		
	16 h[a]	28 h	40 h
Control (C)	4.539 ± 0.576	5.396 ± 0.569	6.147 ± 0.578
Vancomycin (V)	4.696 ± 0.764	3.928 ± 1.378	3.867 ± 2.171
Teicoplanin (T)	4.931 ± 0.808	4.474 ± 0.548	3.798 ± 1.696

CFU, colony-forming units.
[a] End of 16 h incubation time.

Table 2
Number of living animals during the study in each treatment group

Time point	Control	Vancomycin	Teicoplanin
16 h	13	13	13
28 h	10	12	13
40 h	10	11	12

between 20% and 48% in Group T, and was 32% and 47% in the two rabbits in Group V.

4. Discussion

Staphylococcus aureus is the third most common agent in bacterial meningitis in our clinic over 27 years as well as in Turkey [17–20]. MRSA meningitis nearly always develops as a nosocomial infection after neurosurgical operations and the cumulative analysis of series published or presented in congresses shows a mortality rate of 30% [3–5,11,21].

Many MRSA strains are also resistant to several other antibiotics, including all other β-lactam antibiotics, macrolides and lincosamides, whilst usually being highly susceptible only to vancomycin and teicoplanin. In this case, the two glycopeptide agents vancomycin and teicoplanin are the antibiotics of choice [6–8].

To our knowledge, there is no comparative human or animal study comparing teicoplanin and vancomycin in meningitis or MRSA meningitis. Vancomycin usually does not penetrate into the CSF in the absence of inflamed meninges, but when meningitis develops penetration may be enhanced to a moderate degree [22]. Several treatment failures have been reported when vancomycin has been used alone intravenously [2–5]. Although vancomycin may be given via intrathecal application [6], it is frequently administered by intravenous route in the major published MRSA meningitis series [3–5,11]. An additional strategy is to use combination therapy such as vancomycin + rifampicin [6].

There are few papers relating to the use of teicoplanin in MRSA meningitis [9–12]. Teicoplanin has favourable pharmacokinetics, including an extremely long half-life [6]. Stahl et al. [23] measured the CSF levels of teicoplanin in seven non-MRSA meningitis patients. Patients were administered 400 mg intravenous (i.v.) teicoplanin as a single dose on days 2 and 5. CSF sampling was performed at 2 h in two patients, at 4 h in two patients, at 5 h in one patient and at 8 h in two patients. None of the CSF sam ples had a teicoplanin concentration greater than 0.3 mg/L (using a bioassay) except one sample that was obtained at 2 h. These data differ from those obtained from a rabbit experimental model of meningitis [24], in which continuous infusion of 2 mg/kg teicoplanin for 8 h resulted in drug concentrations high enough to allow penetration (3.1 mg/L) of the drug to the inflamed meninges. Kralinsky et al. [9], Cruciani et al. [10] and Venditti et al. [12] treated a total of four cases of MRSA meningitis with intrathecal teicoplanin. The first MRSA meningitis cases treated only with i.v. teicoplanin were reported by Arda et al. [11]. In the study, in which ten cases of MRSA meningitis were reported, six were treated with regimens including i.v. teicoplanin. In two of these six patients it was combined with other agents (one with meropenem and the other with chloramphenicol empirically). None of the patients receiving i.v. teicoplanin or vancomycin had a mortal outcome. Five patients treated with regimens including teicoplanin received the drug as a dose of 2×6 mg/kg. For this reason, the teicoplanin regimen chosen in this study was also 2×6 mg/kg. The 20 mg/kg vancomycin dose was that used in the rabbit models for the treatment of multidrug-resistant pneumococcal meningitis [13,25].

The major methods for measurement of drugs in body fluids are bioassay, high-pressure liquid chromatography (HPLC), fluorescent polarisation study, radioimmunoassay and fluorescent immunoassay [26]. For most drugs, the most sensitive but most expensive method is HPLC [26], but there is no significant difference between these methods for measurement of high concentrations of vancomycin [16]. Bioassay is the most widely used method for both drugs [14–16]. The lowest drug detection limit of bioassay for teicoplanin and vancomycin ranges between 0.25 mg/L and 2.5 mg/L; in our study it was 1 mg/L [14–16,26,27].

The bactericidal effects of vancomycin and teicoplanin are time, not concentration, dependent [28,29]. For this reason, we checked the trough level instead of the peak levels. Peak levels were not measured because of potential mortality of the rabbits and because the main aim of the study was to evaluate the antibacterial effect of the drugs. Fernandez et al. [30] recently compared teicoplanin versus teicoplanin + ceftriaxone in a rabbit meningitis model. Both treatment arms had similar activity. After 15 mg i.v. teicoplanin infusion, the trough CSF teicoplanin level was 0.25 ± 0.17 mg/dL and the trough serum concentration was 6.06 ± 1.43 mg/dL. Our findings of serum drug levels are in concordance with previous findings [28–30].

In our study, four rabbits in the teicoplanin group and two rabbits in the vancomycin group had CSF drug levels higher than the lowest drug detection limit of the bioassay (>1 mg/L) at 40 h. This result may be attributed to i.v. (5 mL) bolus administration of the drug instead of continuous infusion, which was reported to be associated with higher CSF drug levels in an earlier study [24]. Dosage of the drugs might have been inadequate, or a longer time (longer than 24 h, or more than two doses) may be necessary for reaching higher concentrations. Shorter elimination half-lives of teicoplanin and vancomycin (1.7 ± 0.1 h for vancomycin and 7.0 ± 1.0 h for teicoplanin) in rabbits may also have caused low CSF levels [31]. The lower drug detection limit of the bioassay and the absence of peak drug concentrations are additional limitations of our study. The presence of less than 1 mg/L teicoplanin or vancomycin in the CSF of rabbits cannot be excluded [30], but even in such a situation it would probably be inadequate for treatment of the infectious process. A trough teicoplanin concentration greater than 10 mg/L and a trough vancomycin concentration of 5–15 mg/L are suggested in the treatment of severe MRSA infections [28,29]. In our study, a trough CSF drug level greater than 5 mg/L was observed in only one rabbit. A possible post-antibiotic effect of the drugs, or their sub-MIC effect, might have played a role in the rabbits lacking high trough drug levels but having bacterial response [28,29].

Staphylococcus aureus is the third most common bacterial agent encountered in acute purulent meningitis in our

country. To our knowledge, our study is the first to compare teicoplanin and vancomycin, which are basic choices in MRSA meningitis. Our results suggest that teicoplanin is at least as effective as vancomycin in the treatment of MRSA meningitis in an experimental meningitis model in rabbits. Additional data should confirm our experiments in advance of clinical trials to assess efficacy in humans.

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