



# Comparative analysis of linezolid, vancomycin, and hyperbaric oxygen therapies in a rat model of ventriculoperitoneal shunt infection

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Received: 26 November 2023 / Accepted: 27 January 2024 / Published online: 6 February 2024  
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## Abstract

**Purpose** *Staphylococcus epidermidis* is the most common causative microorganism of ventriculoperitoneal shunt infections. This study aimed to compare linezolid and vancomycin treatments and to examine the effect of these antibiotics alone and combined with hyperbaric oxygen therapy on the amount of bacterial colonies in the experimental *S. epidermidis* shunt infection model.

**Methods** A shunt catheter was placed in the cisterna magna of 49 adult male Wistar albino rats. The rats were randomly divided into seven groups, as follows: sterile control, infected control, vancomycin, linezolid, hyperbaric oxygen, vancomycin + hyperbaric oxygen, linezolid + hyperbaric oxygen. In all groups except the sterile control group, 0.2 ml 10<sup>7</sup> CFU/mL *S. epidermidis* was inoculated to the cisterna magna. Parenteral vancomycin was administered 40 mg/kg/day to the vancomycin groups, and 50 mg/kg/day of enteral linezolid to the linezolid groups. Hyperbaric oxygen groups were given 100% oxygen at a pressure of 2.4 ATA for 50 min a day. One day after the last treatment, colony quantities in the shunt catheters and CSF were analyzed.

**Results** The number of CSF colonies in the linezolid group was significantly lower than in the vancomycin group ( $p < 0.05$ ). The number of CSF colonies in the linezolid + HBO group was significantly lower than in the vancomycin + HBO group ( $p < 0.05$ ).

**Conclusions** Linezolid treatment was found to be more effective than vancomycin in ventriculoperitoneal shunt infection caused by *S. epidermidis*. There was no statistical difference among other treatment groups. Hyperbaric oxygen therapy is shown to contribute to the sterilization of cultures.

**Keywords** Ventriculoperitoneal shunt infection · Linezolid · Vancomycin · Hyperbaric oxygen treatment

## Introduction

In contemporary medical practice, hydrocephalus is commonly treated using the ventriculoperitoneal shunt procedure. Cerebrospinal fluid (CSF), which accumulates in the ventricles, is transferred to the peritoneal cavity through

the use of a catheter [1, 2]. Infection is the most common complication of ventriculoperitoneal shunt surgeries. Shunt infection rates vary between 2 and 15% in different series [3–10]. The most common cause of shunt infections is *Staphylococcus epidermidis* (*S. epidermidis*). *S. epidermidis* causes %50 of shunt infections and *S. aureus* causes %25 of shunt infections [11]. Gram-negative bacillies such as *Propionibacterium acnes* and *Enterococcus faecalis* can also cause shunt infection frequently [12]. *S. epidermidis* is frequently encountered in shunt infections since it is common in the skin flora and forms a biofilm. *S. epidermidis* infections are difficult to treat because the immune system is relatively ineffective at clearing biofilm matrix-forming bacteria [11, 13]. Since  $\beta$ -lactam antibiotic resistance is high in *S. epidermidis* infections, intravenous or intraventricular vancomycin is preferred primarily for treatment. Penetration of vancomycin to CSF is almost

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negligible in conditions where the blood–brain barrier is normal but ranges from 7 to 37% in cases of infection [11]. With the emergence of multi-drug-resistant microorganisms, alternative antibiotics have been used in the treatment. Linezolid is the first member of the oxazolidinone group of antibiotics and has %100 oral bioavailability and %90 CSF penetration [14–16]. It is thought to be superior to vancomycin, especially in methicillin-resistant *S. epidermidis* cases [17, 18].

Hyperbaric oxygen therapy (HBO) is a form of treatment administered to the patient or experimental animal by breathing 100% oxygen under a pressure higher than atmospheric pressure [19]. Hyperbaric oxygen therapy increases oxygen tension in the infected tissue, leading to a direct bactericidal effect on certain anaerobic organisms [20]. Additionally, it enhances local microcirculation and promotes leukocyte migration to the infected area, thereby increasing phagocytosis of microorganisms and activating macrophages, resulting in an antibacterial effect [21].

Hyperbaric oxygen is used clinically in the treatment of diabetic wounds, surgical wound infections, deep infections such as osteomyelitis, and prosthetic infections [22]. The contribution of hyperbaric oxygen to antibacterial therapy has been demonstrated in several rat infection models [23–25]. The benefits of hyperbaric oxygen therapy in neurosurgical infections have been demonstrated through various studies [20]. Similarly, in the treatment of brain abscess, hyperbaric oxygen therapy has been shown to reduce the need for repeat surgeries and improve survival rates [26, 27].

In our knowledge, this is the first study in the literature comparing the efficacy of vancomycin and linezolid in shunt infection and evaluating the effectiveness of hyperbaric oxygen therapy in shunt infection.

## Methods

The experiment was carried out in accordance with EU Directive 2010/63/EU for animal. Ethical Committee approval received from Marmara University (29.2020.mar). In this research study, the strain used was *Staphylococcus epidermidis* ATCC 35984, which was acquired from the Department of Microbiology at Marmara University. Initially, the strain was inoculated on 5% sheep blood agar and then incubated at 37 °C in an oven for 24 h. Subsequently, a bacterial suspension was prepared with a density of 0.5 McFarland (equivalent to  $10^8$  CFU/mL) in 0.9% saline by carefully collecting the growing colonies using a loop. The suspension was further diluted ten-fold to achieve a bacterial concentration of  $10^7$  CFU/mL. During the surgical procedure, subjects were inoculated with 0.2 mL of the  $10^7$  CFU/mL bacterial suspension.

## Surgery

In this study, the model defined by Hazer et al. [8] was used as the model of ventriculoperitoneal shunt infection. Rats were anesthetized with intraperitoneal ketamine (100 mg/kg) and xylazine (30 mg/kg). The procedure was performed in the prone position. The surgical area was shaved before the procedure and covered with a sterile drape after proper sterilization. The suboccipital region was opened with a midline vertical incision and the atlantooccipital membrane was exposed. A total of 0.2 cc of CSF was withdrawn from the cisterna magna and 0.2 cc of saline was injected in the sterile group. In other groups, 0.2 cc  $10^7$  CFU/mL *S. epidermidis* was injected. A 5-mm vertical incision was made in the dura, exposing the great cistern. A silicone shunt catheter, one centimeter in length, was implanted into the great cistern, with half of the catheter remaining within the muscle layer.

## Treatment: In vivo rat model

Forty-nine adult male Wistar rats (weight range 270 to 300 g) were used in this study. There were seven rats in each group.

Group 1. Uncontaminated control group: no contamination and no antibiotic therapy was given.

Group 2. Untreated contaminated control group: contamination with *S. epidermidis* was made; no antibiotic therapy was given.

Group 3. Contamination with *S. epidermidis* was made; rats received intraperitoneal 40 mg/kg/day of body weight vancomycin twice a day for 7 days.

Group 4. Contamination with *S. epidermidis* was made; rats received oral 50 mg/kg/d of body weight linezolid twice a day for 7 days.

Group 5. Contamination with *S. epidermidis* was made; rats received HBO at 2.4 absolute atmosphere (ATA) for 50 min once a day for 7 days (Fig. 1).

Group 6. Contamination with *S. epidermidis* was made; rats received intraperitoneal 40 mg/kg/day of body weight vancomycin twice a day and HBO at 2.4 ATA for 50 min once a day for 7 days.

Group 7. Contamination with *S. epidermidis* was made; rats received oral 50 mg/kg/day of body weight linezolid twice a day and HBO at 2.4 ATA for 50 min once a day for 7 days.

## Sample collection

Twenty-four hours after the treatment, all the subjects were euthanized with high-dose anesthetic. The surgical field



**Fig. 1** Rats in the hyperbaric oxygen chamber

was explored under sterile conditions. The CSF sample was taken and the shunt catheter was removed. CSF samples taken and suspensions obtained by vortexing each catheter in 1 ml of physiological saline for 1 min were plated on blood agars using calibrated plastic specials (1  $\mu$ L and 10  $\mu$ L). After the culture plates were incubated for 24 h at 37 °C, colony counts were made. Results were recorded as colony-forming units (CFU/mL).

### Statistical analysis

In the descriptive statistics of the data, mean, standard deviation, median, lowest, highest, frequency, and ratio values were used. Logarithmic transformation was applied to the data. The distribution of variables was evaluated with the Kolmogorov-Smirnov test. Non-parametric tests were used because parametric test conditions were not met. In the analysis of quantitative independent data, the Kruskal-Wallis test was used for multiple group comparisons, and the Mann-Whitney *U* test was used for pairwise group comparisons. SPSS 27.0 program was used in the analysis. The significance level was accepted as  $p < 0.05$ .

## Results

### Catheter cultures

In the sterile control group, no growth was observed in catheter cultures of any of the subjects. However, all subjects in the infected control group showed growth in catheter

**Table 1** Outcome of the catheter cultures

| Group                | Therapy          | Number of culture negative/total | Bacterial count* |
|----------------------|------------------|----------------------------------|------------------|
| Group 1              | No therapy       | 7/7                              | 0.00 $\pm$ 0.00  |
| Group 2 <sup>a</sup> | No therapy       | 0/7                              | 2.62 $\pm$ 2.77  |
| Group 3 <sup>a</sup> | Vancomycin       | 3/7                              | 1.56 $\pm$ 1.71  |
| Group 4 <sup>b</sup> | Linezolid        | 6/7                              | 0.45 $\pm$ 0.87  |
| Group 5 <sup>a</sup> | HBO              | 3/7                              | 2.47 $\pm$ 2.79  |
| Group 6 <sup>b</sup> | Vancomycin + HBO | 4/7                              | 1.58 $\pm$ 1.79  |
| Group 7 <sup>b</sup> | Linezolid + HBO  | 6/7                              | 0.63 $\pm$ 1.05  |

\*Mean  $\pm$  SD log<sub>10</sub> CFU/ml

<sup>a</sup> $p < 0.05$  compared with group 1

<sup>b</sup> $p < 0.05$  compared with group 2

cultures. When comparing the contaminated group with the treatment groups, a significant decrease in colony count was observed in the linezolid, vancomycin + HBO, and linezolid + HBO groups ( $p < 0.05$ ). No significant difference was found among the treatment-administered groups (Table 1).

### CSF cultures

In the sterile control group, no growth was observed in any subjects. In the infected control group, growth was detected in all subjects. When comparing the contaminated group with the treatment groups, significant reductions in colony counts were observed in the linezolid, vancomycin + HBO, and linezolid + HBO groups. The colony count in the linezolid group was significantly lower than in the vancomycin group ( $p < 0.05$ ). In the linezolid + HBO group, the colony count was significantly lower than in the vancomycin + HBO group ( $p < 0.05$ ) (Table 2).

**Table 2** Outcome of the CSF cultures

| Group                  | Therapy          | Number of culture negative/total | Bacterial count* |
|------------------------|------------------|----------------------------------|------------------|
| Group 1                | No therapy       | 7/7                              | 0.00 $\pm$ 0.00  |
| Group 2 <sup>a</sup>   | No therapy       | 0/7                              | 4.03 $\pm$ 4.01  |
| Group 3 <sup>a</sup>   | Vancomycin       | 2/7                              | 2.96 $\pm$ 3.08  |
| Group 4 <sup>bc</sup>  | Linezolid        | 6/7                              | 0.15 $\pm$ 0.57  |
| Group 5                | HBO              | 5/7                              | 3.53 $\pm$ 3.93  |
| Group 6 <sup>ab</sup>  | Vancomycin + HBO | 3/7                              | 2.35 $\pm$ 2.44  |
| Group 7 <sup>bcd</sup> | Linezolid + HBO  | 7/7                              | 0.00 $\pm$ 0.00  |

\*Mean  $\pm$  SD log<sub>10</sub> CFU/ml

<sup>a</sup> $p < 0.05$  compared with group 1

<sup>b</sup> $p < 0.05$  compared with group 2

<sup>c</sup> $p < 0.05$  compared with group 3

<sup>d</sup> $p < 0.05$  compared with group 6

The administration of hyperbaric oxygen therapy, when administered alone or with vancomycin, has demonstrated an augmentation in the quantity of subjects attaining sterilization in cerebrospinal fluid cultures as well as catheter cultures. However, the results are not statistically significant. In essence, while HBO appears to exhibit a potential effect on microbial clearance in CSF and catheter cultures, the observed changes do not reach a level of statistical significance that would unequivocally affirm the efficacy of HBO in this regard.

**Complications** No side effects were observed due to vancomycin and linezolid treatments. Otorrhagia due to barotrauma was observed in one rat in group 5 on the last day of treatment.

## Discussion

The present study aimed to compare the efficacy of vancomycin and linezolid in a ventriculoperitoneal shunt infection model induced by *S. epidermidis* and to explore the potential impact of hyperbaric oxygen therapy. Our investigation yielded significant findings that contribute to the understanding and management of ventriculoperitoneal shunt infections.

In line with previous studies, this study confirmed that enterally administered linezolid exhibited superior efficacy compared to vancomycin in this specific infection model. This outcome aligns with earlier *in vivo* infection models outside the central nervous system, where linezolid has demonstrated favorable results against *S. epidermidis* [17, 18, 27, 28]. Enteral administration of linezolid treatment also suggests that it provides a significant advantage over vancomycin. The efficacy comparison between these antibiotics in a ventriculoperitoneal shunt infection model has not been previously explored. This study is the first *in vivo* study to compare the efficacy between vancomycin and linezolid in shunt infection.

Hyperbaric oxygen therapy contributes to the treatment of spinal infections and cranial infections in neurosurgery [15]. Bartek et al. could manage to treat 12 of 14 patients with neuromodulator implant infections with antibiotics and hyperbaric oxygen therapy without removing the implant [29]. In another study, it was shown that the reoperation rate in patients who received hyperbaric oxygen therapy in addition to antibiotics after surgery for spontaneous brain abscess was lower than in those who did not receive HBO therapy [1]. Yang et al. used hyperbaric oxygen therapy after VP shunt placement to observe clinical improvement in NPH patients. In this study, 31 patients with programmable V-P shunts received HBO treatment. It has been observed that HBO treatment

increases the cognitive performance of patients [30]. The presence of a ventriculoperitoneal shunt, as in the study, is not a contraindication for hyperbaric oxygen therapy. Hyperbaric oxygen therapy can be applied by consulting the shunt manufacturer.

This study aims to investigate the efficacy of hyperbaric oxygen therapy as an adjunct therapy in ventriculoperitoneal shunt infections. Although prior research [29, 31] has demonstrated the utility of hyperbaric oxygen therapy in various neurosurgical infections, there was a noticeable absence of any animal study, case report, or clinical research focusing on hyperbaric oxygen therapy for ventriculoperitoneal shunt infections. Consequently, our study stands as a pioneering endeavor in this area.

While our statistical analysis did not reveal any significant impact of hyperbaric oxygen therapy on colony counts, it did bring to light a crucial observation. Hyperbaric oxygen therapy demonstrated a remarkable ability to sterilize cerebrospinal fluid and catheter cultures, particularly when used alone or in conjunction with vancomycin. This finding indicates the potential for hyperbaric oxygen therapy as a sterilization approach in ventriculoperitoneal shunt infections; further research is needed to elucidate its exact mechanisms and clinical implications.

Despite its contributions, our study faced inherent limitations, mainly related to the ethical considerations limiting the sample size of the animal count. Therefore, we emphasize the necessity for larger-scale animal studies and well-designed clinical trials to validate and extrapolate our findings. These future investigations will provide robust evidence on the therapeutic value of hyperbaric oxygen therapy and potentially revolutionize the management of ventriculoperitoneal shunt infections.

## Conclusion

Our study enriches the scientific knowledge on the comparative efficacy of vancomycin and linezolid in ventriculoperitoneal shunt infections and sheds light on the potential utility of hyperbaric oxygen therapy as an adjunctive treatment. As the first study to explore these aspects in this infection model, our findings serve as a basis for future research efforts aimed at improving the therapeutic effect against ventriculoperitoneal shunt infections.

**Author contribution** Y.Ş.: drafting the article, conception and design, analysis, and interpretation of data, drafting the article, statistical analysis. E.S.: conception and design, analysis, and interpretation of data. Y.A.: administrative, technical, material support. Y.B.: conception and design, critically revising the article, study supervision.

**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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