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EXPERIMENTAL THERAPEUTICS

In Vitro Synergy and *In Vivo* Activity of Tigecycline-Ciprofloxacin Combination Therapy against *Vibrio vulnificus* Sepsis

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ABSTRACT The mortality rate associated with *Vibrio vulnificus* sepsis remains high. An *in vitro* time-kill assay revealed synergism between tigecycline and ciprofloxacin. The survival rate was significantly higher in mice treated with tigecycline plus ciprofloxacin than in mice treated with cefotaxime plus minocycline. Thus, combination treatment with tigecycline-ciprofloxacin may be an effective novel antibiotic regimen for *V. vulnificus* sepsis.

KEYWORDS sepsis, tigecycline, *V. vulnificus*

Vibrio vulnificus is an opportunistic human pathogen that causes skin and soft tissue infections and septicemia. It is introduced by ingestion of contaminated seafood or contact of a wound with seawater (1, 2). Antibiotics used to treat this infection include combination therapy consisting of minocycline plus third-generation cephalosporin and quinolone monotherapy, based on the results of *in vitro* (3) and *in vivo* (4, 5) studies. However, the mortality rate for *V. vulnificus* sepsis remains high (≥50%) despite application of these regimens.

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Tigecycline is the first member of the glycylcycline class of antibiotics and was approved for the treatment of complicated skin and soft tissue infections and intra-abdominal infections, based on noninferiority studies (7, 8). Moreover, tigecycline is active against *Vibrio* species *in vitro*, with a low resistance rate (9). Therefore, tigecycline may be an effective therapeutic agent for *V. vulnificus* infections of skin and soft tissues, including necrotizing fasciitis. However, few *in vivo* studies are available on the therapeutic use of tigecycline for *V. vulnificus* infection (10, 11). In this study, we evaluated the activity of tigecycline-based therapy against *V. vulnificus* infection *in vitro* and *in vivo*.

Two strains clinically obtained from patients with *Vibrio* sepsis, *V. vulnificus* CMCP6 and *V. vulnificus* MO6-24/O, were used in the time-kill study, and *V. vulnificus* CMCP6 was used in animal experiments. The MICs of cefotaxime, minocycline, ciprofloxacin, and tigecycline were measured using the microdilution method (12). To evaluate synergy *in vitro*, a time-kill assay was performed as described previously (13). Cefotaxime (Chong Kun Dang Pharmaceutical, Seoul, Republic of Korea), ciprofloxacin (Ildong Pharmaceutical, Seoul, Republic of Korea), minocycline (Sigma-Aldrich, St. Louis, MO), and tigecycline (Pfizer Korea, Seoul, Republic of Korea) were used in the *in vitro* and *in vivo* animal studies. Antibiotic synergy was defined as a ≥2-$\log_{10}$-CFU/ml decrease at 24 h by combination treatment compared with the most active single antibiotic agent and a ≥2-$\log_{10}$-CFU/ml decrease at 24 h compared with the starting inoculum (14, 15).

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V. vulnificus CMCP6 isolates were incubated overnight at 37°C in a shaking incubator in cation-adjusted Mueller-Hinton broth. A total of 100 μl of the bacterial suspension was transferred to 10 ml of the same fresh broth and incubated for 3 h at 37°C. Bacteria grown were collected by centrifugation, and the pellet was resuspended in 0.85% saline, as described previously (5). For *in vivo* mice experiments, 8-week-old female BALB/c mice (Samtako, Osan, Republic of Korea) with an average body weight of 20 g were used in the study. To establish iron-overload status, 1,000 μg of ferric ammonium citrate was administered intraperitoneally (i.p.) 30 min before *V. vulnificus* inoculation (16). Next, 1×10^8 CFU *V. vulnificus* were inoculated subcutaneously (s.c.) on the right thigh, as described previously (5, 17). The mice were randomly assigned to the treatment groups. All antibiotics were initially given 2 h after the animals were infected. Control mice were treated with 0.1 ml sterile saline i.p. every 6 h, and antibiotics were given for 42 h. The animals were monitored every 6 h over a 48-h period and then every 24 h for another 48 h.

Tigecycline 6.25 mg/kg was administered s.c. every 12 h, in accordance with a recent *V. vulnificus* study in septic mice (10) and another study that measured serum and tissue concentrations and pharmacokinetic parameters of tigecycline (18). The tigecycline dose with the most similar pharmacokinetic effects to the human dose was determined (19). Cefotaxime 180 mg/kg/day i.p. and minocycline 4 to 6 mg/kg/day i.p. were used in most previous studies (4, 5, 17, 20). In this study, to ensure that we did not underestimate the activity of cefotaxime and minocycline, higher doses of cefotaxime (150 mg/kg every 6 h [600 mg/kg/day]) and minocycline (50 mg/kg every 24 h) were given i.p. in accordance with recent mouse *V. vulnificus* infection models (10); these doses corresponded to the maximum human doses in recent pharmacokinetic mouse studies (21, 22). Ciprofloxacin 8 mg/kg was administered i.p. every 12 h as in previous mouse *V. vulnificus* infection models (4, 17, 23, 24), in which similar pharmacokinetics in mice and humans were seen (25, 26). In the survival study, animals were euthanized when they exhibited combined clinical criteria totaling $\geq$8 points. The clinical criteria were defined according to the Korean Food and Drug Administration (KFDA) guidelines as changes in body weight (0 to 3 points), hair coat (0 to 2 points), eye opening (0 to 2 points), activity (0 to 2 points), and posture (0 to 3 points) (27); the higher the score, the worse the condition of the mouse. All experimental mice were housed in a semi-specific pathogen-free (SPF) facility. All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Chonnam National University and KFDA (27). The study protocol was approved by the IACUC of Chonnam National University Hwasun Hospital. The Kaplan-Meier method and log-rank test were used for survival analyses. *P* values of $<$0.05 indicated statistical significance. Statistical analyses were performed using SPSS (v.24.0; SPSS Inc., Chicago, IL) and GraphPad Prism (v. 7.0; GraphPad Software, La Jolla, CA) software.

The MICs of cefotaxime, minocycline, ciprofloxacin, and tigecycline for *V. vulnificus* CMCP6 and MO6-24/O isolates were 0.0625, 0.0625, 0.03, and 0.0625 mg/liter, respectively. Antibiotic synergy was observed *in vitro* in 3/4 MIC time-kill assays for tigecycline-ciprofloxacin after 24 h in *V. vulnificus* strains CMCP6 (Fig. 1a) and MO6-24/O (Fig. 1b). The tigecycline-cefotaxime combination showed a $\geq$2-$\log_{10}$-CFU/ml decrease at 24 h compared with each antibiotic agent used alone but did not meet the criteria for synergy because the bacterial count after treatment decreased by $<$2 $\log_{10}$ CFU/ml compared with the starting inoculum (CMCP6, 5.7×10^5 to 8.0×10^3 CFU/ml; MO6-24/O, 5.7×10^5 to 1.0×10^4 CFU/ml).

Figure 2 presents the survival rates in each treatment group after inoculation with 1×10^8 CFU of *V. vulnificus* CMCP6. The 96-h survival rate was significantly higher in mice treated with tigecycline-ciprofloxacin (71% [17/24]) than in mice treated with cefotaxime-minocycline (42% [10/24]; log-rank test, $P = 0.04$). The 96-h survival rate of the tigecycline-cefotaxime group (67% [16/24]) was higher than that of the cefotaxime-minocycline group, but the difference was not statistically significant (log-rank test, $P = 0.09$). The survival rate of the tigecycline-ciprofloxacin group was also higher than that of the ciprofloxacin (56% [14/25]) and tigecycline (54% [13/24]) monotherapy groups;

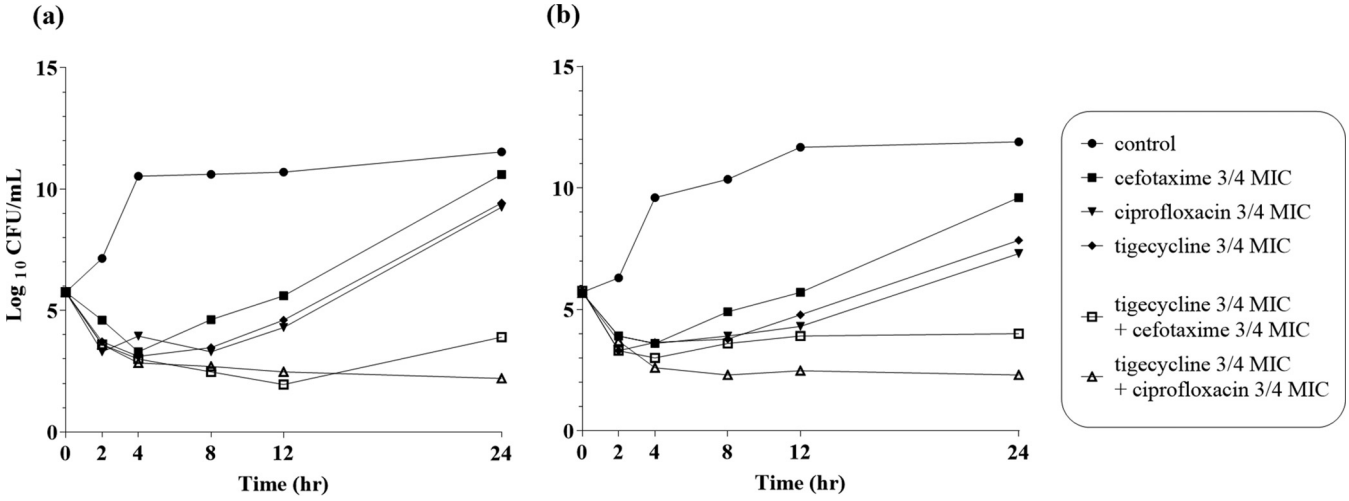

FIG 1 Time-kill curves for *V. vulnificus* CMCP6 (a) and MO6-24/O (b) isolates after incubation with 3/4 MICs of single and multiple antibiotics.

however, the differences were not significant (log-rank test, $P = 0.20$ and 0.29, respectively).

Tigecycline monotherapy may not be appropriate for *V. vulnificus* sepsis because *V. vulnificus* sepsis commonly accompanies bacteremia and tigecycline enters tissues rapidly after administration, resulting in low serum levels. Moreover, a recent study suggested that combination therapy has a better treatment outcome than monotherapy in *V. vulnificus* sepsis (23). Few studies have explored the *in vivo* therapeutic activity of tigecycline-cephalosporin against *V. vulnificus* infection (10). Lin et al. (11) reported the case of a 12-year-old boy administered tigecycline-cefpirome salvage therapy, but the therapeutic activity of this combination cannot be determined by a single case report. Tang et al. (10) showed that a tigecycline-cefotaxime regimen was associated with superior survival compared with cefotaxime-minocycline and tigecycline monotherapy in mice infected with a 1.25×10^6 inoculum of a *V. vulnificus* Vv14-3 clinical isolate. However, *in vitro* synergy of cefotaxime and tigecycline was not seen in 25% (2/8) of the tested isolates. In addition, the therapeutic activity of tigecycline-quinolone remains unknown. In our study, tigecycline-

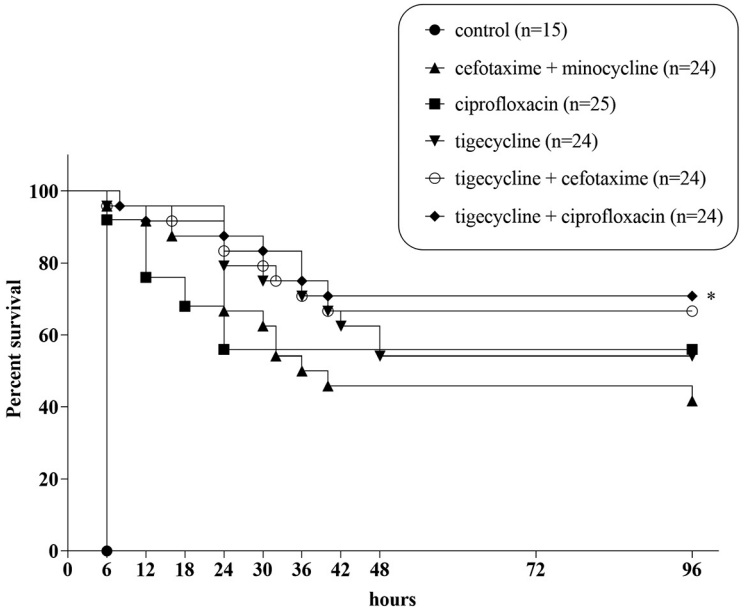

FIG 2 Survival curve of mice infected with 1×10^8 CFU *V. vulnificus* CMCP6. *, $P < 0.05$.

cefotaxime combination failed to show *in vitro* synergy in two reference strains against *V. vulnificus* infection, but the *in vivo* activity was comparable to that of combination treatment with tigecycline-ciprofloxacin. We consistently found that tigecycline-ciprofloxacin showed *in vitro* synergy and was associated with better survival than cefotaxime-minocycline in mice infected with *V. vulnificus*.

In conclusion, our *in vitro* and *in vivo* studies suggest that tigecycline in combination with ciprofloxacin is a potent option for the treatment of invasive *V. vulnificus* infection. Further studies are required to evaluate the activity of tigecycline-ciprofloxacin combination therapy in a clinical setting.

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We have no conflicts of interest to declare.

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