Carbapenem-induced endotoxin release in Gram-negative bacterial sepsis rat models

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Abstract

The carbapenem-induced endotoxin release was evaluated using experimental models of Gram-negative bacterial sepsis in Wistar rats. Infections with Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris and Proteus mirabilis resulted in an increase of the plasma endotoxin concentration after treatment with ceftazidime and carbapenems including imipenem, panipenem, meropenem and biapenem. Except for P. aeruginosa, the plasma endotoxin concentrations after carbapenem treatment were significantly lower than those after ceftazidime treatment. It is noteworthy that treatment of P. aeruginosa sepsis with meropenem or biapenem induced significantly more endotoxin release than other carbapenems and the endotoxin concentrations induced by these carbapenems reached those of ceftazidime treatment. The plasma endotoxin concentrations appeared to correlate with the reduction of platelet counts and the elevation of both glutamic oxaloacetic transaminase and glutamic pyruvic transaminase values.

Keywords: Endotoxin; Antibiotic-induced endotoxin release; Morphological change; Carbapenem; Sepsis model

1. Introduction

The clinical relevance of antibiotic-induced endotoxin release in the course of treatment for Gram-negative bacterial sepsis has been reported [1–3]. A number of studies have revealed considerable variation among antibiotics in their propensity to release endotoxin from bacteria during their antibacterial action [4–12]. Jackson and Kropp [7] provided experimental evidence which suggested that differences in the cumulative binding affinity of the penicillin-binding protein (PBP) for individual β-lactam antibiotics were related to the propensity of releasing endotoxin from Gram-negative bacilli in vitro.

A number of in vivo studies have presented data indicating that antibiotic treatment can cause an increase in the plasma endotoxin concentration [3,5,8,11,13] and that released endotoxin may be
detrimental to the host with the Gram-negative bacterial infection [13,14]. Opal et al. [11] showed a significant difference between ceftazidime and imipenem in the plasma concentration of endotoxin released after antibiotic treatment in the *Escherichia coli* and *Pseudomonas aeruginosa* sepsis rat models. These results provided supportive evidence that differences in the plasma endotoxin concentration may reflect the mode of antibacterial action of the antibiotics. However, some studies have reported that there was no difference in the plasma endotoxin concentration between control and antibiotic-treated rats [3].

In this report, we developed experimental models of Gram-negative bacterial sepsis in rats for *E. coli*, *Serratia marcescens*, *Klebsiella pneumoniae*, *P. aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis* and evaluated the plasma endotoxin concentration after carbapenem treatment.

### 2. Materials and methods

#### 2.1. Bacterial strains, media and culture conditions

Six clinical isolates including *E. coli*, *S. marcescens*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris* and *P. mirabilis* were used in the present study. Carbapenem-induced morphological changes of these strains were studied previously by scanning electron microscopy [15]. Each strain showed typical morphological changes among clinical isolates tested. Bacteria were stored at −70°C in Luria-Bertani (LB) broth (Difco Laboratories, Detroit, MI, USA) containing 20% glycerol, inoculated on LB agar plates and incubated at 37°C overnight for use.

#### 2.2. Antibiotics

The antibiotics used were ceftazidime (Japan Glaxo, Tokyo, Japan), imipenem (Banyu Pharmaceutical, Tokyo, Japan), panipenem (Sankyo, Tokyo, Japan), meropenem (Sumitomo Pharmaceutical, Osaka, Japan) and biapenem (Lederle Japan, Tokyo, Japan).

#### 2.3. In vivo endotoxin release study

The MIC values of the antibiotics used in this study for the different bacteria were shown in Table 1. Wistar male rats (225–235 g), purchased from Japan SLC (Hamamatsu, Japan), were challenged with an intraperitoneal inoculation of 10⁶ c.f.u. of each bacterial culture. After 24 h, the rats were administered an intravenous dose of antibiotics at 0.5× MIC or a saline solution as the placebo control. There was no mortality within 24 h of the study. After 4 h, blood (10–12 ml) was drawn by aortic puncture for the endotoxin assay, a complete blood count (CBC), biochemical assays and quantitative bacteriology. Blood samples were collected in endotoxin-free glass test tubes. An aliquot of each blood sample was used for the determination of the CBC, biochemical assays and quantitative bacteriology. Blood samples were collected in endotoxin-free glass test tubes. An aliquot of each blood sample was used for the determination of the CBC (Sysmex K-800, Toa-Iyo-Denshi, Kobe, Japan) and quantitative bacteriology, and the plasma was immediately prepared at 4°C by centrifugation at 800×g for 10 min. At least two animals were used in each group. All samples were stored at −70°C until analysis. The plasma levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and creatinine (Cr) were determined using GOT-UV test-wako, GPT-UV test-wako and Creatinine test-wako (Wako Pure Chemical Industries,

### Table 1

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg ml⁻¹) for:</th>
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<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
</tr>
<tr>
<td>Panipenem</td>
<td>0.125</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.032</td>
</tr>
<tr>
<td>Biapenem</td>
<td>1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Osaka, Japan), respectively. Quantitative bacterial cultures were measured by serial dilution of blood in endotoxin-free physiological saline and spread plating on LB agar. The detection limit of the quantitative bacterial cultures was $10^1$ c.f.u. ml$^{-1}$.

2.4. Endotoxin assay

Glass test tubes were heated at 250°C for 3 h to ensure that they were endotoxin-free. Sterile endotoxin-free plastic specimen containers and pipette tips were used for all endotoxin assays. The activity of endotoxin was determined by a chromogenic endotoxin-specific assay using the Limulus coagulation enzyme (ES test, Seikagaku-Kogyo, Tokyo, Japan). Before the endotoxin assay, endotoxin concentrations were measured by passing plasma samples across 0.45-μm filters. The endotoxin concentrations were calculated by comparison with the reference endotoxin, E. coli O111:B4 LPS. The samples were serially diluted as needed in endotoxin-free physiological saline (Otsuka Pharmaceutical, Tokyo, Japan) to achieve concentrations in the range 0.01–0.5 ng ml$^{-1}$, within which there is a linear relationship between E. coli endotoxin O111:B4 concentration and spectrophotometric absorbance.

2.5. Statistical analysis

Results of plasma endotoxin concentrations are expressed as mean ± S.E. Paired data were compared using a two-tailed paired $t$-test. $R^2$ coefficients for the primary correlation were determined using Microsoft Excel ver. 5.0. $P$ values of $<0.05$ were considered significant.

3. Results

3.1. Plasma level of endotoxin released from bacteria after carbapenem treatment

The plasma endotoxin concentration of an experimental model of Gram-negative sepsis following the treatment with carbapenems and ceftazidime is shown in Fig. 1. The viable cells of each sample were $10^2$–$5 \times 10^3$ c.f.u. ml$^{-1}$ following 4 h exposure to antibiotics. Except for P. aeruginosa, the plasma

![Fig. 1](https://example.com/fig1.png)

Fig. 1. The plasma endotoxin concentrations (ng ml$^{-1}$) released from E. coli, S. marcescens, K. pneumoniae, P. aeruginosa, P. vulgaris and P. mirabilis following 4 h treatment with each antibiotic: 1, none; 2, ceftazidime; 3, imipenem; 4, panipenem; 5, meropenem; and 6, biapenem. Data are the plasma endotoxin concentrations ± S.E.
endotoxin concentrations after treatment with each carbapenem were significantly lower than those with imipenem and panipenem (each $P < 0.05$), reaching those levels with ceftazidime treatment. Antibiotics themselves have no effect on the Limulus assay (data not shown).

3.2. Determination of complete blood count and plasma enzyme values

The decrease in the platelet count had a negative correlation with the increase in the plasma endotoxin concentration for each strain (Fig. 2A, $R^2 = 0.48$, $R^2 = 0.73$, $R^2 = 0.67$, $R^2 = 0.71$, $R^2 = 0.88$ and $R^2 = 0.66$ for E. coli, S. marcescens, K. pneumoniae, P. aeruginosa, P. vulgaris and P. mirabilis, respectively). Other parameters of the CBC such as white blood cell counts, red blood cell counts, and hemoglobin had no correlation with the plasma endotoxin concentration (data not shown). The increases in the values of GOT and GPT had positive correlations with the increase in the plasma endotoxin concentration for each strain (Fig. 2B,C, $R^2 = 0.80$ and $R^2 = 0.88$, $R^2 = 0.79$ and $R^2 = 0.39$, $R^2 = 0.88$ and $R^2 = 0.75$, $R^2 = 0.96$ and $R^2 = 0.52$, $R^2 = 0.85$ and $R^2 = 0.32$, and $R^2 = 0.85$ and $R^2 = 0.88$, respectively). The Cr values showed no correlation with the plasma endotoxin concentration (data not shown). The correlation of the GOT value with the plasma endotoxin concentration was stronger than the other parameters investigated under our experimental conditions.

4. Discussion

To date, several studies have been carried out using animal models to evaluate the antibiotic-induced endotoxin release from Gram-negative bacilli [8,9,11,13,16–18]. These studies have demonstrated antibiotic-induced endotoxin release in vivo. We also confirmed that infection of rats with E. coli, S. marcescens, K. pneumoniae, P. aeruginosa, P. vulgaris and P. mirabilis followed by treatment with various carbapenems and ceftazidime resulted in increases in the plasma free-endotoxin concentration.

Except for P. aeruginosa, the carbapenem treatment resulted in less plasma endotoxin release than the ceftazidime treatment. In P. aeruginosa sepsis, however, the plasma free-endotoxin concentrations...
following treatment with meropenem or biapenem were significantly higher than those with imipenem and panipenem, reaching concentrations as high as those after ceftazidime treatment. This is contrary to some previous findings that imipenem, panipenem and biapenem generally induced much less plasma endotoxin release than meropenem and ceftazidime from various Gram-negative bacteria in vivo [19].

We have previously demonstrated that the endotoxin release in vitro after carbapenem treatment correlated with the type of morphological changes [15]. Except for *P. aeruginosa* treated with meropenem and biapenem, carbapenems induced formation of spherical or ovoid cells [15]. *P. aeruginosa* exposed to meropenem and biapenem were filamentous cells with a ‘bulge’ midway along the cells [15]. Ceftazidime used as a control induced filamentous formation [15]. As shown in our in vitro study, there were significant differences in the plasma free endotoxin concentrations between bacteria; one formed spheroplasts and the other formed filamentous cells with or without a ‘bulge’ midway along the cells. That is, the combination of bacteria and antibiotics, which resulted in filament induction, was associated with release of a large amount of plasma endotoxin, whereas another combination, which resulted in spheroplast induction, caused a small amount of plasma endotoxin release.

Additionally, our results showed that both the GOT and GPT values showed a positive correlation with the plasma free-endotoxin concentration, while the platelet count showed a negative correlation, and the Cr value showed no correlation. Finally, we must stress that some combinations of bacterial sepsis and antibiotics, even carbapenems, can induce release of the large amount of endotoxin in vivo.

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References