Teicoplanin reduces in-vitro reactivity and murine lethality of *Salmonella minnesota* R595 lipopolysaccharide

A. Focà, G. Matera*, M. C. Berlingieri*, M. C. Liberto and G. B. De Sarro*

*Institute of Microbiology; Institute of Pharmacology, University of Reggio Calabria, Policlinico ‘Mater Domini’, 88100 Catanzaro, Italy

Three different tests were performed to investigate the effect of teicoplanin on lipopolysaccharide (LPS). After incubation for 3 h with teicoplanin, LPS from *Salmonella minnesota* R595 showed reduced reactivity in the metachromatic dimethyl-methylene blue assay and the limulus amoebocyte lysate test. In addition, galactosamine-sensitized mice had an increased survival rate, from 29% to 72%, when teicoplanin was pre-incubated for 3 h with the LPS to be injected intraperitoneally. The results suggest that teicoplanin may have a neutralizing effect on LPS.

Introduction

Patients suffering from bacteraemia may worsen following clinical use of bactericidal antibiotics to which infecting bacteria are sensitive (Shenep *et al.*, 1988). Antibiotic-induced liberation of substantial amounts of endotoxin from Gram-negative organisms has been observed in experimental in-vitro (Cohen & McConnell, 1986) and in-vivo models. It has also been measured sequentially during human infections and correlated with clinical outcome (Shenep *et al.*, 1988). Antibiotics with an anti-LPS activity may, therefore, be useful to control endotoxin-dependent sepsis sequelae induced by different Gram-negative infections.

Artenstein & Cross (1989) described the reduction of endotoxin (lipopolysaccharide; LPS) activity by aminoglycosides. Our recent data are basically consistent with those of Artenstein & Cross (1989), although obtained with a different experimental approach (Focà *et al.*, 1991). Furthermore, in the course of screening for antimicrobials which neutralize LPS, the glycopeptide antibiotic teicoplanin was found to inhibit *Salmonella minnesota* R595 LPS, both in vitro and in vivo.

Artenstein & Cross (1989) evaluated the anti-endotoxin activity of antibiotics in microtitre wells by the inhibition of limulus amoebocyte lysate (LAL) test. However, an endotoxin assay with the metachromatic dye 1,9-dimethyl-methylene blue (DMB) has been reported to be reproducible, specific, positively correlated to LPS toxicity, and less laborious and expensive than other endotoxin tests. The DMB assay was used to evaluate in-vitro reactivity of *S. minnesota* R595 LPS by Focà *et al.* (1991).

Polymyxin B binds to lipid A, the toxic moiety of LPS, and reduces or abrogates most of the biological activities and chemical properties of endotoxin, including in-vitro reactivity in the LAL test and in the DMB assay; however, clinical use of polymyxin B has been hampered by many major side effects (Artenstein & Cross, 1989).
In contrast, teicoplanin has been used at a dosage as high as 14.4 mg/kg/day in humans and was well tolerated (Martino et al., 1989).

The aim of this study was to examine the LPS-neutralizing ability of teicoplanin by comparing antibiotic-LPS mixtures with antibiotic-free LPS in the DMB assay and the LAL test. The effects on the lethality of LPS in galactosamine-sensitized mice were also studied.

Methods

LPS from *S. minnesota* R595 (Calbiochem, San Diego, USA), teicoplanin (Lepetit, Milano, Italy) and polymyxin B sulphate (Pfizer, New York, USA) were all dissolved in sterile water for injection. Further dilutions were made in sterile water for in-vitro assays, and in sterile saline for in-vivo experiments. 1,9-Dimethyl-methylene blue was used to prepare DMB reagent for the LPS metachromatic assay as described previously (Focà et al., 1991).

Serial dilutions (10, 33, 100, 333, 1000 mg/L) of tested antibiotics were incubated with 500 mg/L of LPS in a sterile 96-well microtitre plate at 37°C, unless otherwise stated. After incubation for 3 h, a 0.1 mL sample was taken from each well, mixed with DMB reagent and immediately read at 535 nm. The OD_{535} reading of an antibiotic plus sterile water sample was subtracted from the OD_{535} reading of each antibiotic plus LPS sample. The resulting values were averaged and compared with averaged OD_{535} readings of antibiotic-free LPS samples.

A commercially available chromogenic LAL test (Coatest; Kabi, Nyköping, Sweden) has been reported to be c. 10^7 times more sensitive than the DMB assay. Therefore, LPS (50 pg/mL) was incubated at 37°C in a sterile 96-well microtitre plate with serial dilutions of antibiotic (10, 33 or 100 pg/mL) in order to reproduce the three highest antibiotic/LPS ratios used during the DMB assay. After 3 h, 0.1 mL was taken from each well and diluted with 0.2 mL of sterile water. The LAL test was performed following the instructions of the manufacturer, but the results were not corrected for the dilution factor. OD_{405} readings were corrected as described above in the DMB assay.

Ten week old male C57BL/6 mice (Charles River, Italy) were sensitized to endotoxin effects by intraperitoneal (ip) injection of 8 mg D-galactosamine hydrochloride. One hour later, each mouse was injected ip with 100 ng of LPS, either alone or pre-incubated for 3 h with 666 ng of polymyxin B or 2000 ng of teicoplanin. Control animals were treated with either LPS alone, antibiotic alone, or saline alone. Data were expressed as the mean ± s.e. and were subjected to analysis of variance. Fisher's PLSD test was used to determine significant differences between groups.

Results

Table I compares the effects of either teicoplanin or polymyxin B dilutions on LPS (500 mg/L) reactivity with DMB reagent. Table II shows the reduction of LPS reactivity in the LAL test by teicoplanin. The addition of polymyxin B (10 pg/mL) to LPS resulted in significant neutralization of LPS in this test (data not shown).

Galactosamine-sensitized mice treated with LPS alone had a survival rate of 29% (*n* = 7). When the same dose of LPS was inoculated after pre-incubation for 3 h with teicoplanin, the survival rate increased to 72% (*n* = 7). A mixture of LPS plus polymyxin B produced a survival rate of 75% (*n* = 8), while control animals injected with either antibiotic alone (*n* = 7) or with saline alone (*n* = 7) showed no deaths.
Effect of teicoplanin on LPS

Table I. Effect of teicoplanin and polymyxin B on metachromatic reactivity (OD\textsubscript{530}) of \textit{S. minnesota} R595 LPS (500 mg/L) with DMB reagent. The data are the mean results±S.E. of at least five experiments

<table>
<thead>
<tr>
<th>Antibiotic concentration (mg/L)</th>
<th>Reactivity (OD\textsubscript{530})</th>
<th>teicoplanin</th>
<th>polymyxin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.213±0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.197±0.008</td>
<td>0.214±0.005</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0.190±0.011</td>
<td>0.166±0.008</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.182±0.008</td>
<td>0.127±0.009</td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>0.169±0.009</td>
<td>0.075±0.016</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>0.156±0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 vs antibiotic-free control LPS by Fisher's PLSD test.

Discussion

The results obtained in these experiments indicate that teicoplanin has the ability to neutralize LPS. The LPS used in the present study consists only of 2-keto-deoxyoctonic acid plus lipid A, the toxic and more conserved part of LPS. Lipid A is normally found deep inside the bacterial outer membrane, but is thought to become available to interact adversely with host cells following LPS liberation by antibiotics (Cohen & McConnell, 1986). Thus, the results might hold true for interaction between teicoplanin and LPS released from many different Gram-negative organisms.

Shenep \textit{et al.} (1988) recently quantified the course of bacteraemia and endotoxaemia in antibiotic-treated bacteraemic patients from before the start of antimicrobial therapy. A significant increase in the plasma level of free LPS was found, and this correlated with a worse clinical outcome (Shenep \textit{et al.}, 1988). The same authors suggested that, during both Gram-positive and Gram-negative severe infections, gut-derived LPS may pass from portal to systemic circulation because of impairment of reticuloendothelial cells (Shenep \textit{et al.}, 1988). Similarly, non-lethal experimental endotoxaemia may produce a self-promoted translocation of bacteria and their endotoxins from the intestinal flora (Deitch \textit{et al.}, 1989).

Teicoplanin is a glycopeptide antibiotic introduced recently for the therapy of

Table II. Reactivity of LPS (50 pg/mL) and teicoplanin combinations, as measured at OD\textsubscript{405} by a chromogenic limulus amoebocyte lysate test. The data are the mean results±S.E. of three experiments

<table>
<thead>
<tr>
<th>Teicoplanin concentration (pg/mL)</th>
<th>Reactivity (OD\textsubscript{405})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.671±0.061</td>
</tr>
<tr>
<td>10</td>
<td>0.604±0.024</td>
</tr>
<tr>
<td>33</td>
<td>0.355±0.021*</td>
</tr>
<tr>
<td>100</td>
<td>0.282±0.018*</td>
</tr>
</tbody>
</table>

*P < 0.05 vs teicoplanin-free control LPS by Fisher's PLSD test.
Gram-positive infections. However, glycopeptides have also been reported to be effective against Gram-negative cocci (Goldstein et al., 1987) and against deep rough mutants of Gram-negative bacilli (Shlaes et al., 1989) whose LPS has a major lipidic moiety when compared to LPS from smooth strains of Gram-negative bacilli. Following antibiotic-induced cell lysis and exposure of lipid A, the fatty acid tails of teicoplanin may have some physicochemical interaction with the lipid moiety of LPS, similar to the lipid/lipid interaction reported between polymyxin B and lipid A.

The results suggest that the use of teicoplanin in combination with bacteriolytic anti-Gram-negative antibiotics, particularly during empirical chemotherapy, may achieve a broader antibacterial spectrum and afford protection against endotoxic shock sequelae consequent to antibiotic-released LPS.

References


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