Endothelial cell compatibility of glycopeptide antibiotics for intravenous use

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The use of human venous endothelial cells for testing antibiotic solutions for intravenous compatibility provides a valuable alternative to animal models. In order to evaluate the effect of vancomycin and teicoplanin on the viability of human umbilical venous endothelial cells, intracellular ATP levels were measured by a luciferin–luciferase assay. Prostacyclin (PGI₂) and thromboxane A₂ (TXA₂) were determined by direct radioimmunoassay. Vancomycin at concentrations of 5 and 10 mg/mL reduced the intracellular ATP content by 18.7% and 69.9%, respectively, within 60 min. In contrast, cellular energy charge remained significantly higher after incubation with teicoplanin at 5 and 10 mg/mL (reduction 8.7% and 15.5%, respectively). Neither vancomycin nor teicoplanin at a concentration of 2 mg/mL led to significant ATP decline. However, endothelial cells incubated with vancomycin resulted in significantly lower release of PGI₂ and TXA₂ compared with teicoplanin. These results show that teicoplanin is more compatible with endothelial cells than vancomycin, and that both antibiotics are well tolerated if diluted to a final concentration of 2 mg/mL.

Introduction

Although glycopeptide antibiotics are generally well tolerated, vancomycin can cause local phlebitis at the site of infusion. Our aim was to investigate whether glycopeptides are directly toxic for endothelial cells, resulting in subsequent phlebitis. To test the tolerance of intravenously applied antibiotics without using animal models, we set up an in-vitro culture system using human umbilical venous endothelial cells (HUVEC). We tested the effects of intravenous vancomycin and teicoplanin. Cell viability was analysed by measuring intracellular ATP levels. Thromboxane (TXA₂) and prostacyclin (PGI₂) production were used as parameters for cell function.

Materials and methods

Cell culture

Endothelial cells were prepared from human umbilical veins. Cells were isolated and cultured according to a modified standard procedure. The confluent primary monolayers (c. 8 × 10⁶ cells/flask) were washed and trypsinized. The cell suspensions were transferred into each well of a 12-well culture plate and cultivated for 4 days. The cells were identified as endothelial cells by their typical ‘cobblestone’, contact inhibited morphology and by production of von Willebrand factor.

Antibiotics

Commercially available preparations of vancomycin hydrochloride (Eli Lilly, Indianapolis, IN, USA) and teicoplanin (Merrell Dow Pharma GmbH, Rüsselsheim, Germany) were dissolved in water for injection and diluted further with 0.9% NaCl for the experiments.

Incubation with vancomycin and teicoplanin

The culture medium was removed and the cell layers were gently washed with Dulbecco’s phosphate-buffered saline (Gibco, Paisley, UK). Thereafter, vancomycin hydrochloride and teicoplanin solutions were added to the endothelial cells at final concentrations of 2.5 and 10 mg/mL. The cells were then incubated for 20, 60 or 120 min in a humidi-
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ified incubator at 37°C with 5% CO₂. The supernatants were transferred into micro-test tubes containing indomethacin (30 μmol/L final concentration) to avoid further eicosanoid formation at the end of each incubation, and were stored in the presence of indomethacin at −70°C.

Measurement of PG₁₂ and TXA₂

Direct radioimmunoassays (BIOTECX) were carried out to determine the concentrations of 6-ketoprostaglandin F₁₅ (6-keto-PGF₁₅) and thromboxane B₂ (TXB₂), the stable degradation products of PG₁₂ and TXA₂, respectively. No 6-keto-PGF₁₅ or TXB₂ could be detected in the incubation medium before addition to the cell layers. The specificity and reliability of the assay for PG₁₂ and TXA₂ was confirmed using the cyclooxygenase inhibitor indomethacin.⁵

Measurement of ATP

The intracellular ATP content was determined in a cell lysate prepared by adding 300 μL of 0.5 M HClO₄ to each well after removal of the culture supernatant. The lysate was neutralized with 1 M K₂CO₃ and centrifuged, then the supernatant was analysed for ATP by means of bioluminescence using an ATP monitoring reagent and an Autolumat 953 luminometer (Berthold, Germany).

Statistical analysis

Data from 12 different representative experiments are expressed as mean ± s.d. The statistical significance was determined using the Mann–Whitney U-test. P values of <0.001 were considered significant.

Results

Effects of vancomycin on HUVEC

Incubation of cells with 5 and 10 mg/mL vancomycin resulted in a rapid decrease in intracellular high energy phosphates to 5.8 ± 0.73 nmol/10⁶ cells and 0.95 ± 0.53 nmol/10⁶ cells, respectively, after 120 min (Figure 1). Vancomycin at concentrations of 2 mg/mL did not lead to significant decline in intracellular high energy phosphates (11.2 ± 0.75 nmol/10⁶ cells) after 120 min. The synthesis of 6-keto-PGF₁₅ and TXB₂ in endothelial cells was not significantly different from that in controls (Figure 2).

Effects of teicoplanin on HUVEC

Teicoplanin at concentrations of 5 and 10 mg/mL reduced the intracellular ATP levels to 9.3 ± 0.69 nmol/10⁶ cells and 8.6 ± 0.68 nmol/10⁶ cells respectively (Figure 1). A dose of 2 mg/mL did not lead to ATP decline. At concentrations of 5 and 10 mg/mL we measured a statistically significant increase of 6-keto-PGF₁₅ production to 53.9 ± 1.5 pmol/10⁶ cells and 86 ± 7.1 pmol/10⁶ cells, respectively, after 60 min. Upon incubation with 10 mg/mL teicoplanin we observed significant TXA₂ release, of 20.7 ± 9.3 pmol/10⁶ cells after 1 h (Figure 2).

Figure 1. Effects of vancomycin and teicoplanin on intracellular ATP levels. Columns show ATP levels after 20 min ( ), 60 min (□) and 120 min (■) (mean ± s.d.). *, Significantly different from controls (P < 0.001).
Glycopeptide antibiotics and endothelial cells

Discussion

We show that commercially available preparations of glycopeptides for intravenous application are well tolerated by endothelial cells when applied in concentrations of 5 mg/mL. Since the antibiotics tested are administered at maximal concentrations of 10 mg/mL, the dose range used in our in-vitro experiments (5 and 10 mg/mL) mimics the clinical concentrations that might be found at the site of infusion. Similar concentrations may be reached by retrograde intravenous pressure infusion techniques. We have demonstrated that these high concentrations lead to considerable endothelial cell damage. These findings may explain the common side-effect associated with intravenously applied glycopeptides, namely pain and phlebitis at the site of infusion. Perhaps the low pH of the vancomycin solutions applied (pH 3.7–4.5) accounts for the cellular dysfunction observed. This might be supported by the observation that cyclooxygenase and other enzymes involved in PG\(_I_2/TX\_A_2\) production are critically pH-dependent. In contrast, teicoplanin (pH 7.2) induces an increase in PG\(_I_2\) and TX\(_A_2\) release from HUVEC at concentrations of 5 and 10 mg/mL. It might be speculated that teicoplanin at these concentrations leads to an increase in inositol triphosphate followed by an elevation of intracellular free calcium and therefore to increased eicosanoid production.

Figure 1 shows that a detrimental effect measurable after 20 min occurs only using vancomycin solutions at concentrations of 10 mg/mL, whereas dilution to 5 mg/mL renders the solutions less harmful to HUVEC. These data are in line with the observation that slow intravenous application of glycopeptides into large veins can largely prevent the occurrence of local phlebitis. Alternatively, it

Table. pH and osmolarity of the commercially available iv preparations of vancomycin and teicoplanin diluted with 0.9% NaCl

<table>
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<th>Vancomycin concentration (mg/mL)</th>
<th>pH</th>
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should be possible to avoid the occurrence of phlebitis by diluting commercial preparations to ≤2–5 mg/mL.

As previously shown for fluoroquinolones, our HUVEC model for testing antibiotic solutions for intravenous compatibility provides a valuable alternative to animal models. In conclusion, our data suggest that commercial preparations of teicoplanin are more compatible with HUVEC than preparations of vancomycin.

References


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