Influence of Vancomycin Infusion Methods on Endothelial Cell Toxicity

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Peripheral intravenous therapy is frequently used in routine hospital practice and, due to various factors, its most common side effect is phlebitis. The infusion of vancomycin is particularly associated with phlebitis despite its widespread use. French guidelines recommend central intravenous infusion for high concentrations of vancomycin, but peripheral intravenous therapy is often preferred in intensive care units. Methods of vancomycin infusion are either intermittent infusion or continuous infusion. A comparison of these methods under in vitro conditions simulating clinical use could result in better infusion efficacy. Human umbilical vein endothelial cells (HUVECs) were therefore challenged with clinical doses of vancomycin over a 24- to 72-h period using these infusion methods. Cell death was measured with the alamarBlue test. Concentration-dependent and time-dependent vancomycin toxicity on HUVECs was noted with a 50% lethal dose at 5 mg/ml after 24 h, reaching 2.5 mg/ml after 72 h of infusion, simulating long-term infusion. This toxicity does not seem to be induced by acidic pH. In comparing infusion methods, we observed that continuous infusion induced greater cell toxicity than intermittent infusion at doses higher than 1 g/day. The increasing use of vancomycin means that new guidelines are required to avoid phlebitis. If peripheral intravenous therapy is used to reduce infusion time, along with intermittent infusion, vein irritation and localized phlebitis may be reduced. Further studies have to be carried out to explore the causes of vancomycin endothelial toxicity.

Intravenous (i.v.) therapy is one of the most common routine medical practices performed in hospitals, due to the increasing complexity of disease processes. Numerous complications, including phlebitis, thrombophlebitis, extravasations and infections are however associated with peripheral i.v. (p.i.v.) therapy. Phlebitis is the most common side effect and may occur at rates ranging from 30 to 70% (1–4), depending on various factors. Infusion phlebitis is an inflammation of the vein endothelium without infection and is characterized by pain, redness, swelling, and warmth at the i.v. site. Phlebitis is a real public health problem, due to the risk of thrombophlebitis, which increases pain and prolongs hospital stays. A large number of risk factors have been implicated in the onset of phlebitis: gender, site of catheter insertion, catheter materials, infusion rates, place of treatment (emergency or hospital), frequency of infusion, and the drug infused (2–5). Studies have revealed the potential endothelial toxicity of some i.v. antibiotics, such as amoxicillin and aminoglycosides (5), erythromycin and dicloxacillin (6), fusidic acid (7), and vancomycin (8, 9).

Vancomycin is an old but powerful antibiotic widely used in industrial countries. With the increasing prevalence of methicillin-resistant Staphylococcus aureus, Clostridium difficile, and nosocomial infections due to the Staphylococcus species, more patients require vancomycin therapy. With this increased use of vancomycin, nurses report a higher incidence of p.i.v. complications (8, 9), especially as vancomycin requires long-term therapy. The general toxicity (e.g., nephrotoxicity and otoxicity) of vancomycin is well known, as well as the “red man syndrome,” generally associated with the rapid administration of vancomycin (<30 min) and characterized by pruritus, erythema of the face, neck, and upper torso and in severe cases, angioedema and cardiovascular collapse (10). Nevertheless, local vancomycin toxicity has not yet really been studied. The acidity of a vancomycin solution (pH < 4) is often accepted as a cause of complications, but other causes may exist. Indeed, Roszell and Jones (8) have shown that the administration of vancomycin involves more complications (i.e., due to the number of venipunctures, infiltrations, etc.) than that of other antibiotics with a low pH.

The methods for vancomycin infusion vary worldwide. Intermittent infusion is still the reference method, while continuous infusion is increasingly used, especially in France. Both of these methods are effective. Studies have analyzed the continuous i.v. infusion method, which is increasingly used to obtain a higher vancomycin serum level and steadier target concentration (11–13) compared to the conventional method, i.e., intermittent infusion. However, no improved drug activity and no change in the course of the disease has been demonstrated with either method (14, 15). Indeed, Wysocki et al. found that the microbiological and clinical results of the two methods were similar (16). French
The rate of vancomycin-induced phlebitis through intermittent infusion varies from 0 to 18% for a known concentration of 4 to 5 mg/ml over 1 h (17, 18). As for continuous infusion, Farber and Moellerling, using an infusion of 4 mg/ml over 24 h, reported a phlebitis rate of 13% (19). Clinical studies comparing continuous and intermittent vancomycin infusion offer very limited data on the rate of vancomycin-induced phlebitis (11, 16, 20). Since vancomycin is being used increasingly, it is essential to assess the impact of administration methods and the mechanism of its local toxicity in order to avoid associated complications. A comparison of different infusion methods should result in an improvement in vancomycin infusion, as well as a reduction in endothelial vancomycin toxicity.

The aim of the present study was to assess vancomycin endothelial cell toxicity under in vitro conditions simulating clinical use to determine the factors implicated in local vancomycin toxicity.

**MATERIALS AND METHODS**

**Drugs.** Vancomycin (500 mg/10 ml; Mylan, France) was reconstituted in saline solution (0.9% NaCl; Viaflo, Baxter, France) and diluted in culture medium (Promocell GmbH, Heidelberg, Germany) cultured in endothelial cell growth medium (Promocell GmbH) enriched with endothelial cell growth supplement mix (Promocell GmbH), streptomycin (0.1 g/liter) and penicillin (100 IU/ml), at 37°C in a CO₂ incubator (CB 150/APT line/binder; LabExchange, Paris, France) with 5% CO₂–95% atmosphere and 100% relative humidity. One day before adding the antibiotic solution, the cells were placed in a 96-well plate at a density of 3 × 10⁴ cells/well to establish an 80% confluent monolayer the next day. The culture medium in each well was then replaced by the tested drug solution prepared at different concentrations. The culture plate was further incubated for 24 h. A 100% cellular viability control made with 0.9% NaCl. Each test was performed in triplicate.

**Cell culture.** Tests were performed with proliferating human umbilical vein endothelial cells (HUVECs; Promocell GmbH, Heidelberg, Germany) cultured in endothelial cell growth medium (Promocell GmbH) enriched with endothelial cell growth supplement mix (Promocell GmbH), streptomycin (0.1 g/liter) and penicillin (100 IU/ml), at 37°C in a CO₂ incubator (CB 150/APT line/binder; LabExchange, Paris, France) with 5% CO₂–95% atmosphere and 100% relative humidity. One day before adding the antibiotic solution, the cells were placed in a 96-well plate at a density of 3 × 10⁴ cells/well to establish an 80% confluent monolayer the next day. The culture medium in each well was then replaced by the tested drug solution prepared at different concentrations. The culture plate was further incubated for 24 h. A 100% cellular viability control was made with 0.9% NaCl. Each test was performed in triplicate.

**Cell test protocols.** To assess toxicity related to concentration and exposure time (i.e., contact time between vancomycin and cells), as well as simulating average adult doses, we tested vancomycin concentrations from 1 to 10 mg/ml over 24 h and from 0.5 to 7.5 mg/ml over 24, 48, and 72 h. To assess the absence of any impact of pH variations on cell toxicity, the pH of vancomycin solutions at 5 and 7.5 mg/ml diluted in culture medium at 50/50 (vol/vol) was measured (SympHony Meter, VWR, France), and the 0.9% NaCl solution in culture medium 50/50 (vol/vol) was acidified with hydrochloric acid to reach the same pH. The test was carried out over 24 h.

To compare continuous infusion and intermittent infusion from 1 to 2.75 g/day, we used a vancomycin solution at fixed concentrations ranging from 1.5 to 5 mg/ml for continuous infusion, and from 4 to 11.5 mg/ml in two 1-h periods for intermittent infusion.

**Cell vitality assay.** After a 24-h culture, the cell reaction to different antibiotic solutions was evaluated by fluorometric assay with nontoxic alamarBlue dye (Interchim Montluçon, France), a redox indicator; this is a very simple and versatile means for measuring cell proliferation and cytotoxicity (21). Briefly, the culture medium in each well was replaced by 200 μl of culture medium supplemented with 10% (vol/vol) alamarBlue dye. After a 2-h incubation, 150 μl of reacted dye from each well was transferred into a 96-well Fluoro-LumiNunc plate (Polylabo, Strasbourg, France), and the fluorescence intensity was measured by a Twinkle LB970 fluorometer (Berthold Technology, GmbH & Co. KG, Bad Wildbad, Germany) with an excitation at 560 nm and an emission at 590 nm. The results were expressed as a percentage of viable cells compared to 100% control made with 0.9% NaCl.

**Statistical tests.** Nonparametric tests were used to compare the percentages of surviving HUVECs with the null hypothesis that there is no difference between the experimental conditions assessed. The Mann-Whitney U test was used to assess concentration-dependent toxicity and the influence of pH. The Kruskal-Wallis test was used to assess time-dependent toxicity and to compare continuous and intermittent infusion. In the presence of a significant P value (P < 0.05), an analysis using the Conover and Iman method was made to detect significant differences between couples of contact time. Each of these tests was performed with XLSTAT software version 2012.2.01 (Addinsoft, Paris, France).

**RESULTS**

**Endothelial cell toxicity of vancomycin infusion at a clinical dose.** HUVECs were challenged with clinical doses of vancomycin and cell death was measured. We observed the concentration-dependent toxicity of vancomycin on HUVECs. The results showed a significant increase in HUVEC death from a vancomycin concentration of 2.5 mg/ml on. Indeed, the 50% lethal dose (LD₅₀) of vancomycin on HUVECs reached 5 mg/ml after 24 h of treatment (Fig. 1).

**Endothelial toxicity of long-term vancomycin infusion at a clinical dose.** We observed local toxicity to be also time dependent with significantly higher cell death after 48 and 72 h of treatment than after 24 h (Fig. 2). From 2.5 mg/ml on, vancomycin significantly increased HUVEC death after 48 h and even further after...
Moreover, intermittent infusion induced lower dose-dependent toxicity than intermittent infusion at doses higher than 1 g/day, the results showed that continuous infusion induced more toxicity under test intermittent infusion and continuous infusion to compare different concentrations under various conditions. We decided to perform results similar to those obtained with an animal model (22). Some hypotheses remain regarding vancomycin toxicity. In postsurgical intensive care units, vancomycin is frequently infused along with other i.v. antibiotics from the same Y-site infusion device. In our laboratory, flow variations in p.i.v. infusion during multi-infusion therapy through a single i.v. access have induced variations in drug concentration (26, 27). Disturbances in infusion flow by hydration or other i.v. medication could also influence vancomycin exposure to endothelial cells and so modulate toxicity. A limitation to our method is that it does not reproduce fluid dynamics during infusion. A dynamic cell test mimicking flow variations over 24 h of vancomycin infusion should be performed to connect flow variation and cell toxicity.

Other antibiotics, such as erythromycin or clarithromycin, have been associated with the appearance of proinflammatory cytokines (e.g. interleukin-8 [IL-8] or IL-6) and the upregulation of endothelial receptors involved in inflammatory response (25). It might be interesting to test whether vancomycin could induce such expressions of inflammation markers on HUVECs in long-term infusion and multidrug infusion conditions. An inflammatory response could also be induced by reactive oxygen species (ROS), as indeed vancomycin has already been associated with ROS production in renal cells, inducing nephrotoxicity (28, 29). ROS-induced necrosis could therefore be associated with vancomycin-induced endothelial cell toxicity. The production of vancomycin-induced ROS and/or proinflammatory cytokines by HUVECs will have to be evaluated with the intermittent and/or continuous methods to validate this hypothesis.

Moreover, vancomycin is incompatible with numerous other drugs, especially through Y-site administration. The use of saline flush before and after each dose of incompatible medicines is required to avoid contact with vancomycin. The cell toxicity of vancomycin coinfused with other drugs has to be analyzed to determine the phlebitis risk of such associations. Drug incompatibility is a physical or chemical phenomenon resulting in a concentration-dependent precipitation or a pH alteration. The acidic pH of vancomycin might be modified during the blending process, altering the stability of the drug and possibly causing precipitation (30). Studies have shown that i.v. antibiotics, such as vancomycin, amphotericin B, and especially β-lactam have been associated with a 2-fold increase in the risk of phlebitis, which may be attributed to the presence of microparticles in the antibiotic solution (31, 32) capable of inducing an inflammatory response in

**DISCUSSION**

Our results confirm the local toxicity of vancomycin under clinical conditions with an LD₅₀ of about 5 mg/ml for a 24-h continuous infusion, as described in previous studies (22, 23). To discern cell toxicity induced by low pH, an acidified mixture of NaCl solution (0.9%) was prepared in culture medium. It was observed that pH was not responsible for vancomycin toxicity under test conditions, in conformity with a previous study (8). Since vancomycin is infused over a long period postoperatively, we tested vancomycin toxicity on HUVECs for 48 and 72 h. The results showed that the local toxicity of vancomycin was not only concentration dependent but also time dependent. To be more precise, the LD₅₀ was reduced by half after 48 and 72 h compared to 24 h. In our study, the clinical use of vancomycin was simulated at different concentrations under various conditions. We decided to test intermittent infusion and continuous infusion to compare their toxicity on HUVECs. The results showed, with the same daily dose of vancomycin, that continuous infusion induced greater endothelial toxicity than intermittent infusion did.

The availability of HUVECs to test drug solutions for i.v. compatibility is a valuable alternative to animal models, as demonstrated by some studies that have analyzed antibiotic compatibility and inflammatory processes on HUVECs (24, 25). In particular, Robibaro et al. used HUVECs to mimic a clinical dose of vancomycin at the infusion site in an intermittent infusion model and obtained results similar to those obtained with an animal model (22). Some hypotheses remain regarding vancomycin toxicity. In postsurgical intensive care units, vancomycin is frequently infused along with other i.v. antibiotics from the same Y-site infusion device. In our laboratory, flow variations in p.i.v. infusion during multi-infusion therapy through a single i.v. access have induced variations in drug concentration (26, 27). Disturbances in infusion flow by hydration or other i.v. medication could also influence vancomycin exposure to endothelial cells and so modulate toxicity. A limitation to our method is that it does not reproduce fluid dynamics during infusion. A dynamic cell test mimicking flow variations over 24 h of vancomycin infusion should be performed to connect flow variation and cell toxicity.

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**FIG 3** Cell viability of HUVECs after 24 h of contact with low-pH solution. Percentage of HUVECs surviving a 24 h contact with vancomycin solution at 5 mg/ml (pH 7.15) or 7.5 mg/ml (pH 6.96), compared to 0.9% NaCl solution at the same pH. *, P < 0.05.

**FIG 4** Viability of HUVECs after 24 h of contact with vancomycin at fixed concentrations ranging from 1.5 to 5 mg/ml for continuous infusion and with two 1-h infusions of vancomycin solution at fixed concentrations ranging from 4 to 11.5 mg/ml for intermittent infusion corresponding to daily doses from 1 to 2.75 g. *, P < 0.05.
HUVECs and inducing cell apoptosis. A study has already associated the systemic inflammatory response syndrome (SIRS) with the presence of microparticles (33). The use of a filter for longer term vancomycin infusion could reduce this potential risk, although it may be the catheter material itself that contributes to the risk of phlebitis. Indeed, polyurethane catheters seem to be responsible for a 30 to nearly 50% reduction in the incidence of phlebitis compared to those made of tetrafluoroethylene-hexafluoropropylene (Teflon) (34).

Blood dilutes and neutralizes vancomycin, so reducing vein irritation. Since the blood flow of a central vein is more powerful, the Infusion Nursing Standards of Practice has recommended using a central catheter for vancomycin infusion at concentrations higher than 5 mg/ml to avoid phlebitis or other complications (35). However, a p.i.v. catheter is more frequently used in clinics for vancomycin infusion because of its practicality. We suggest that new guidelines for the p.i.v. infusion of vancomycin should be published to help nurses make an informed choice. Indeed, current recommendations fail to include long-term vancomycin infusion, and studies have shown that phlebitis usually occurs after 24 h of treatment (3).

In conclusion, the increasing use of vancomycin makes new recommendations essential to prevent phlebitis. If a peripheral vein is used to reduce infusion time and limit coinfusion on the same line, the choice of intermittent infusion may reduce vein irritation and localized phlebitis. Nevertheless, this model is an in vitro model and more studies have to be carried out to gain insight into vancomycin toxicity, such as clinical trials comparing intermittent and continuous methods of vancomycin infusion.

REFERENCES


