Endothelial Cell Compatibility of Clarithromycin for Intravenous Use

HARALD VORBACH,1 GUENTER WEIGEL,2 BRUNO ROBIBARO,3 CHRISTINE ARMBRUSTER,1 REINER SCHAUMANN,4 MANFRED HLOUSEK,4 MICHAEL REITER,4 ANDREA GRIESMACHER,2 and APOSTOULOS GEORGOPOULOS4

1Department of Internal Medicine II, Pulmonary Center, 2Department of Cardiothoracic Surgery, 3Department of Pulmonary Medicine, and 4Department of Infectious Diseases, University Hospital of Vienna, Vienna, Austria

Objectives: Tolerance of intravenously applied clarithromycin has been tested on marginal ear veins of rabbits. Use of human umbilical venous endothelial cells (HUVEC) for testing antibiotic solutions for intravenous compatibility provides a valuable alternate model.

Design and methods: In order to evaluate the effect of clarithromycin on intracellular purines, reflecting cell viability, energy production, signal transduction and DNA/RNA synthesis, intracellular adenosine 5' triphosphate (ATP), adenosine 5' diphosphate (ADP), guanosine 5' triphosphate (GTP), and guanosine 5' diphosphate (GDP) levels were measured by means of high performance liquid chromatography (HPLC).

Results: Incubation of cells with 2 mg/mL clarithromycin resulted in a rapid decrease of the intracellular ATP from 12.6 ± 1.1 to 8.87 ± 0.82 nmol/million cells or 1.5 ± 0.6 nmol/million cells, after 20 or 60 min, respectively. In addition, ADP was extensively depleted. Purine nucleotide profiles were markedly different following exposure to 1 mg/mL clarithromycin. There was no significant decline of intracellular high energy phosphate levels after 20 min.

Conclusion: These results show that clarithromycin has a better endothelial compatibility if diluted to a final concentration of 1 mg/mL. These data are in line with our clinical observations that the occurrence of phlebitis could be minimized by diluting the manufacturers' preparation of clarithromycin to 1 mg/mL. Copyright © 1998 The Canadian Society of Clinical Chemists

KEY WORDS: endothelial cells; clarithromycin; antibiotics; phlebitis; ATP; ADP; GDP; GTP.

Introduction

Clarithromycin (6-O-methyl-erythromycin A) is a 14-membered macrolide antimicrobial agent widely used for treatment of infections caused by Chlamydia, Mycoplasma, Legionella, Helicobacter pylori, and Mycobacterium avium complex. It is generally well tolerated with a low incidence of systemic adverse events. Local irritation, predominantly in the gastrointestinal tract and veins, however, may interfere with therapy (1).

The tolerance of intravenously applied clarithromycin has been tested in a study with rabbits utilizing injections of 2 mL/kg of 7.5, 15, and 30 mg of base/mL into the marginal ear vein over 25 to 35 min. The ear veins collected 22 to 23 h following infusion revealed only mild but dose-related venous irritation (2). To provide an alternate test system, we designed an in vitro culture system using human umbilical venous endothelial cells (HUVECs). We tested the effects of clarithromycin available for intravenous application on these cultures. By determining the intracellular contents of adenine and guanine nucleotides using the highly sensitive high performance liquid chromatography (HPLC) method, intact endothelial metabolism was examined. We measured the intracellular adenosine 5' triphosphate (ATP) and adenosine 5' diphosphate (ADP) levels reflecting energy production of these cells. In addition, guanosine 5' triphosphate (GTP) and guanosine 5' diphosphate (GDP), which have an important role for DNA/RNA synthesis, G-protein coupled signal transduction and glycosylation of membrane proteins.

Materials and methods

Cell culture

Endothelial cells were prepared using human umbilical veins. Cells were isolated and cultured according to a modified standard procedure (3,4). Only cells from first subcultures were used for the experiments. The cells were identified as endothelial cells by the typical cobblestone, contact inhibited morphology, by von Willebrand Factor (F VIII: vWF) production and the presence of Weibel-Pallade bodies (5).
ANTIBIOTICS

The commercially available preparation of 500 mg clarithromycin (Abbott, IL, USA) were dissolved in 10 mL distilled water and additionally diluted with 0.9% sodium chloride for the experiments.

INCUBATION WITH CLARITHROMYCIN

For the experiments the culture medium was removed and the cell layers were gently washed with Dulbecco’s phosphate buffered saline (Gibco, Paisly, Scotland). Thereafter, clarithromycin in final concentrations of 2, 1, and 0.5 mg/mL was added to the endothelial cells and incubated for 20 and 60 min. Control experiments were performed using 0.9% NaCl. All incubations were carried out in a humidified incubator at 37° C and 5% CO2.

DETERMINATION OF INTRACELLULAR PURINE NUCLEOTIDES

Purines were measured by means of HPLC. ATP, ADP, GTP, and GDP were separated injecting 100 mL of the neutralized supernatant onto a CNU-010 column (Chemcon; Vienna, Austria) using a KH2PO4 gradient. Buffer A consisted of 0.015 mol/L KH2PO4 (pH 3.45), buffer B of 0.5 mol/L KH2PO4 (pH 3.45). A linear gradient rising from 0% B to 100% B in 40 min was used with a total run time of 60 min and an equilibrium delay of 8 min. The flow rate was 1.2 mL/min and the detection was made at 254 nm (6). The amounts of ATP, ADP, GTP, and GDP formed were determined by the ratio of the peak areas in relation to the corresponding standards measured under the same conditions. The linear range for all four nucleotides was between 0.75 and 30 mmol/L. The results are given as nmol/106 cells.

STATISTICAL ANALYSIS

Data from 12 different experiments are expressed as mean ± SD. The statistical significance was determined by means of Mann–Whitney U-Test; p < 0.001 (*) was considered to be significant.

Results

EFFECTS OF 2 MG/mL CLARITHROMYCIN ON HUVEC

Incubation of cells with 2 mg/mL clarithromycin resulted in a rapid decrease of the intracellular ATP to 8.87 ± 0.82 nmol/million cells or 1.5 ± 0.6 nmol/million cells, after 20 or 60 min, respectively (Figure 1). In addition, ADP was extensively decreased indicating a depletion of the ATP/ phosphocreatine (CP) regeneration system. Although, GTP and GDP were maintained for 20 min during exposure to 2
mg/mL clarithromycin, these nucleotides extensively declined after 60 min (Table 1).

**Effects of 1 mg/mL Clarithromycin on HUVEC**

Purine nucleotide profiles were significantly different during exposure to 1 mg/mL clarithromycin. There was no significant decline of intracellular high energy triphosphates and the corresponding diphosphates after 20 min. On incubation with 1 mg/mL clarithromycin the ATP/ADP and GTP/GDP decrease after 60 min was less pronounced (Table 1).

**Effects of 0.5 mg/mL Clarithromycin on HUVEC**

HUVEC exposed to 0.5 mg/mL clarithromycin for periods up to 60 min showed no significant difference in the purine nucleotide profiles compared to controls, indicating that no cellular damage occurred.

**Discussion**

*In vitro* studies with cultured human cells have proved to be a promising method for predicting toxicity and for clarifying mechanism of toxicity (7,8). As previously shown for fluoroquinolones (4) and glycopeptide antibiotics (9), our *in vitro* model of human venous endothelial cells for testing antibiotic solutions for intravenous compatibility provides an alternate to animal models. For investigating cellular impairment during incubation with clarithromycin, intracellular purine content was measured. As shown in Figure 1, 1 mg/mL clarithromycin led to an ATP decline when compared to untreated HUVEC. This decrease, when combined with the data given in Table 1, can be considered reversible, since ATP levels can be regenerated by the action of creatine kinase using ADP as substrate and phosphocreatine as phosphate donor. Clarithromycin, 2 mg/mL, leads to an ATP depletion that does not seem to be reversible via the creatine phosphate (CP)/ADP regeneration system because of the strong decrease of intracellular ADP. The depletion of intracellular GTP and GDP might support this consideration and reflect a functional and structural alteration.

Because the antibiotic tested is administered at maximal concentrations of 2 mg/mL, the dose range used in our *in vitro* experiments (0.5 mg/mL–2 mg/mL) mimics possible clinical concentrations at the site of infusion.

Table 1 shows that a detrimental effect measurable after 20 min occurs only using clarithromycin solutions at concentrations of 2 mg/mL, whereas already a dilution to 1 mg/mL renders the solutions more compatible to HUVEC. These data are in line with our clinical observation that intravenous application of clarithromycin at concentrations of 1 mg/mL can largely prevent the occurrence of local phlebitis. Other authors provided evidence that thrombophlebitis secondary to intravenous administration of erythromycin was reduced in incidence or severity by reducing the drug concentration in the infusate (10). Our *in vitro* data prove that for clarithromycin the same has to be considered. The importance of this *in vitro* study should be underlined since, first, no data exists on the influence of clarithromycin on human endothelial cells and, second, aside from erythromycin, clarithromycin is the only macrolide preparation for intravenous use available in most European countries.

In conclusion, our data clearly show that intravenously applied clarithromycin has a better endothelial compatibility if diluted to 1 mg/mL.

**References**


