Effect of norepinephrine on cefpirome tissue concentrations in healthy subjects

Ilka M. Steiner¹, Herbert Langenberger¹, Claudia Marsik¹, Bernhard X. Mayer¹, Milena Fischer¹, Apostolos Georgopoulous³, Markus Müller¹, Gottfried Heinz⁴ and Christian Joukhadar¹,²*

¹Department of Clinical Pharmacology, Division of Clinical Pharmacokinetics; ²Institute of Pharmacology; ³Department of Internal Medicine I, Division of Infectious Diseases and Chemotherapy; ⁴Department of Internal Medicine II, Division of Intensive Care Medicine, University of Vienna Medical School, Allgemeines Krankenhaus; Waehringer Guertel 18–20, A-1090 Vienna, Austria

Received 6 October 2003; returned 17 November 2003; revised 28 November 2003; accepted 13 December 2003

Objectives: To test whether norepinephrine (NOR) affects tissue microcirculation and impairs plasma-to-tissue equilibration of antimicrobial agents.

Materials and methods: Eight healthy male volunteers were enrolled to an analyst-blinded, randomized, two-period two-sequence crossover study. A single intravenous dose of 2 g of cefpirome was administered simultaneously with starting a continuous infusion of NOR (0.16 µg/kg per min) or placebo (PL) over 180 min. The microdialysis technique was used for the assessment of unbound cefpirome concentrations in skeletal muscle tissue and subcutaneous adipose tissue. Free plasma concentrations were related to corresponding tissue concentrations. Haemodynamics were determined by the measurement of mean arterial blood pressure (MAP), heart rate and forearm blood flow (FBF).

Results: Area under the concentration–time-curve (AUC) values of cefpirome for interstitium and plasma were not significantly different between the PL and NOR groups (P > 0.47). Tissue penetration of cefpirome as described by the ratios of the AUCs from 0 to 180 min for tissue to the AUC values for plasma were 0.81 ± 0.34 for the PL group and 0.80 ± 0.26 for the NOR group (P > 0.05). Baseline values of MAP, heart rate and FBF were not significantly different between study days. MAP increased significantly following NOR administration from 73.3 ± 3.5 mmHg at baseline to 94.0 ± 5.2 mmHg during infusion (P = 0.017). NOR exerted no significant effects on FBF.

Conclusions: We have shown that intravenous administration of NOR does not exert a significant effect on peripheral blood flow and tissue penetration of cefpirome in healthy men. This might be attributed to systemic regulatory mechanisms, which probably fully compensate for major changes in blood flow in peripheral tissues.

Keywords: microdialysis, forearm blood flow, haemodynamics, pharmacokinetics

Introduction

Critically ill patients frequently suffer from severe bacterial infections leading to sepsis or septic shock and inadequate organ perfusion. This necessitates immediate therapeutic intervention, which is originally based on the restoration of fluid volume and the intravenous administration of potent vasopressors. In these cases, vasopressor therapy with catecholamines, mostly with norepinephrine (NOR), is initiated. In low doses, NOR causes distinct vasoconstriction and increases mean arterial blood pressure (MAP) without causing deterioration of vital organ function. In doses frequently necessary in septic shock patients, NOR causes general constriction of capillary beds in peripheral tissues and significantly impairs blood flow in skeletal muscle and subcutaneous adipose tissue.

Blood flow, however, was recently shown to be a major determinant of drug distribution within body compartments. Pharmaceutical agents such as antimicrobials have to pass the capillary endothelial barrier to equilibrate with the interstitium of peripheral tissues. Therefore, recent clinical trials have hypothesized that the administration of NOR is one potential explanation for low concentrations of antibiotics in target tissues in critically ill patients. This
Effect of norepinephrine on cefpirome tissue concentration

is of particular clinical relevance, because microbial persistence is likely to occur, and the emergence of bacterial resistance is triggered by concentrations of antimicrobial agents below the minimal inhibitory concentration (MIC) value of the causative pathogen.6,7 Critically ill patients may, therefore, be at increased risk for therapeutic failure.

In this study, we aimed to test the hypothesis that administration of NOR markedly affects tissue distribution of antimicrobials. Cefpirome, a fourth generation cephalosporin was used as a model compound. The rationale for selecting cefpirome was based on the fact that its plasma and tissue pharmacokinetics are well documented in patients and healthy volunteers.5,9 The well-established microdialysis technique was used for the measurement of antimicrobial concentrations in interstitial space fluid of soft tissues.10

Materials and methods

The study protocol was approved by the Ethics Committee of the University of Vienna, Medical School. The study was carried out in accordance with the Declaration of Helsinki (1964) in the revised version of 1996 (Somerset West), the Guidelines of the International Conference of Harmonization (ICH), the Good Clinical Practice (GCP) Guidelines, and Austrian Drug Law (Arzneimittelgesetz). Healthy volunteers were enrolled in the study after their written informed consent was obtained. They received a detailed description of the study before any examination or intervention was undertaken.

Volunteers

Eight Caucasian healthy male volunteers with a mean age of 26.6 ± 5.3 years, a mean body mass index of 22.3 ± 1.1 kg/m² and a mean weight of 71.1 ± 4.9 kg were enrolled in the study.

In an initial screening visit, they were physically checked; blood pressure and ECG parameters were recorded. Normotension was demanded for inclusion, defined as systolic blood pressure (SBP) < 130 mmHg and diastolic blood pressure (DBP) < 85 mmHg after a 5 min rest in the supine position. Venous fasting blood samples were taken for analysis of complete blood count, laboratory chemical parameters and for hepatitis and HIV serology tests. Volunteers were questioned about past medical history and treatment. The subjects were enrolled in the study unless the investigator considered abnormal values in blood chemistry and physical examination relevant. Subjects were non-smokers and they had to be drug free for at least 3 weeks before the first study day.

Sample size calculation

The sample size calculation was carried out based on the equation published by Stolley & Strom.13 A sample size of eight subjects with paired measurements has 80% power to detect differences of approximately 15% in AUC values9 and arterial blood flow10 between groups.

Study design

A randomized, analyst-blinded, placebo-controlled, two-period, two-sequence, crossover study was carried out. Volunteers were randomly assigned to receive NOR on study day one or on study day two. Wash out period was at least 1 week. Microdialysis and forearm blood flow measurements were carried out on both study days.

During study days, subjects were in a supine position. Three plastic cannulas were inserted into cubital and antecubital veins for drawing blood samples and for the administration of cefpirome combined with NOR or cefpirome combined with placebo (PL).

Cefpirome (Cefrom, Albert Roussel Pharma, Vienna, Austria) was administered as an intravenous single dose of 2 g over a period of 10 min on both study days.

Norepinephrine (Arterenol, Hoechst AG, Frankfurt, Germany) was administered by a primed infusion of step-wise increasing doses for periods of 10 min per dose step. Thereafter, NOR was administered continuously over 180 min at a rate of 0.16 µg/kg per min.

Ringer’s solution (Ringer Lösung ‘Mayrhofer’ Infusionslösung, Mayrhofer Pharma Gesellschaft m.b.H., Linz, Austria) served as placebo.

Microdialysis

For the determination of free interstitial concentrations of cefpirome, in vivo microdialysis was carried out.10-12 This method is based on sampling of analytes from the interstitial space by means of a semi-permeable membrane at the tip of a microdialysis probe. Once the microdialysis probe is implanted into the tissue, substances present at a certain concentration (Ctissue) in the interstitial fluid diffuse out of the extravascular fluid into the probe, resulting in a concentration (Cdialysate) in the perfusion medium. For most analytes, equilibrium between interstitial space fluid and the perfusion medium is incomplete (Ctissue > Cdialysate). The factor by which the concentrations are interrelated is termed relative recovery.

For calibration of the microdialysis (MD) probes, in vivo recovery was assessed in each experiment according to the retrodialysis method.15 The principle of this method relies on the fact that diffusion through a semi-permeable membrane is a bidirectional process and quantitatively equal. Therefore, cefpirome was added to the perfusate at a concentration of 30 mg/L.11

The in vivo recovery value was calculated as:

\[
\text{Recovery} (%) = 100 - \left(100 \times \frac{\text{concentration}_{\text{dialysate}}}{\text{concentration}_{\text{perfusate}}}\right)
\]

Commercially available microdialysis probes (CMA 10, Microdialysis AB, Stockholm, Sweden) with a molecular weight cut-off of 20 kDa, an outer diameter of 0.5 mm, and a membrane length of 16 mm were inserted into subcutaneous adipose tissue and skeletal muscle of the thigh. Before probe insertion, the skin was cleaned and disinfected. The surface of the skin was punctured by 20-gauge intravenous plastic cannulas without anaesthesia. The steel trochars were removed and the MD probes were inserted into the tissues via use of guidance cannulas. The plastic cannula was removed, leaving the probe under the surface of the skin. Then the MD probes were connected to microinfusion pumps (CMA 100, Stockholm, Sweden or Precidor; Infors-AG, Basle, Switzerland) which provided a constant flow-rate of 1.5 µL/min throughout the study period. Two baseline samples at an interval of 15 min were collected during MD probe calibration. The whole MD system was manually rinsed after the termination of MD probe calibration. Then the MD probes were re-connected to the microinfusion pumps and were constantly perfused with Ringer’s solution for a period of approximately 30 min. Finally, a sample was collected over 15 min immediately before cefpirome and NOR or PL administration was started. Thereafter, the sampling interval was extended to 20 min. All samples were subjected to chemical analysis. There was no detectable cefpirome in any sample collected before cefpirome administration.

Blood sampling

Following MD probe calibration, samples of dialysates and venous blood were drawn at 20 min intervals over a period of 180 min. Samples were kept on ice for a maximum of 30 min until centrifugation. The venous catheter was rinsed with Ringer’s solution after each sampling. Blood samples were centrifuged at 4°C, 2500g for 5 min; cells were discarded and plasma was obtained. Plasma and dialysate samples were frozen at −80°C until analysis.

507
Measurements of blood pressure and heart rate
Systolic and diastolic blood pressure and heart rate (HR) were measured in 30 min intervals on both study days.

Forearm blood flow measurements
Forearm blood flow (FBF) was measured by venous occlusion plethysmography. The principle of the technique has been described previously. All measurements were carried out using a mercury-filled silicone strain-gauge plethysmograph (Hokanson EC6 Plethysmograph, Bellevue, WA, USA). The rate of forearm swelling during venous occlusion is related to arterial inflow. Venous outflow of the forearm was abruptly stopped by inflating an occlusion cuff above the elbow to a pressure of 50 mmHg. This pressure was sufficient to occlude the veins while not affecting arterial inflow. The relationship between raising forearm volume and arterial inflow is only valid if the veins are not distended. Therefore, the forearm was placed at the level of the right atrium. The rate of swelling is a measure of total arterial blood flow. In all subjects, the time to reach C max, t1/2β = the terminal elimination half-life; V = apparent volume of distribution; CL = apparent total body clearance; ND = not determined.

Main PK parameters are presented in Table 1. The concentration–time profiles of cefpirome were identical for subcutaneous adipose and muscle tissue in both groups. In addition, no significant differences (P > 0.05) in PK parameters of cefpirome was found between groups for AUC0–180min, tmax, and t1/2β in plasma and interstitium. In addition, no significant differences (P > 0.05) in V and CL values were detected between groups. The concentration versus time profiles of cefpirome were identical for subcutaneous adipose and muscle tissue in both groups.

Tissue penetration parameters are presented in Table 2. Tissue penetration as described by the ratios of AUC0–180 for muscle to AUC0–180 for plasma and AUC0–180 for subcutaneous adipose to AUC0–180 for plasma were not significantly different for the NOR and the placebo groups (P > 0.05).

Results of MD probe calibration
In vivo recovery values for skeletal muscle tissue were 17.2 ± 7.9% in the NOR group and 17.7 ± 5.6% for the PL group. For subcutaneous adipose tissue, recovery values were 19.5 ± 11.7% in the NOR group and 19.2 ± 7.6% in the PL group.

Table 1. Main pharmacokinetic data

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th></th>
<th>Muscle</th>
<th></th>
<th>Subcutis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>placebo</td>
<td>NOR</td>
<td>placebo</td>
<td>NOR</td>
<td>placebo</td>
<td>NOR</td>
</tr>
<tr>
<td>AUC0–180min (mg·min/L)</td>
<td>14715 ± 4894</td>
<td>15482 ± 4773</td>
<td>11044 ± 2140</td>
<td>12191 ± 4291</td>
<td>1278 ± 4427</td>
<td>11207 ± 2889</td>
</tr>
<tr>
<td>Cmax (mg/L)</td>
<td>188 ± 82</td>
<td>208 ± 81</td>
<td>131 ± 32</td>
<td>139 ± 69</td>
<td>144 ± 70</td>
<td>118 ± 32</td>
</tr>
<tr>
<td>tmax (min)</td>
<td>23 ± 8</td>
<td>20 ± 0</td>
<td>40 ± 0</td>
<td>43 ± 8</td>
<td>51 ± 6</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>t1/2β (min)</td>
<td>85 ± 18</td>
<td>84 ± 21</td>
<td>74 ± 24</td>
<td>100 ± 48</td>
<td>96 ± 19</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>V (L)</td>
<td>13.4 ± 3.5</td>
<td>13.3 ± 6.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CL (L/min)</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

All data are presented as mean ± S.D.; AUC = area under the concentration–time curve; Cmax = the maximum concentration of cefpirome; tmax = the time to reach Cmax; t1/2β = the terminal elimination half-life; V = apparent volume of distribution; CL = apparent total body clearance; ND = not determined.

Statistical analysis
Statistical analysis was carried out using commercially available software (Statistica, StatSoft, Inc., Tulsa, OK, USA). As parameters were non-normally distributed, Wilcoxon matched pairs tests were used for comparison between study groups. Analyses of variance (Friedmann-ANOVA) for repeated measurements were used. A P value < 0.05 was considered the level of significance. All data are presented as mean ± standard deviation (S.D.).

Results
One subject suffered from strong headache immediately after the start of NOR administration and decided to stop participation in the study. Thus, data sets from only seven volunteers were eligible for PK and FBF analysis.

The plasma protein binding of cefpirome was approximately 10%. All plasma data presented in the manuscript relate to the unbound fraction of cefpirome.
Effect of norepinephrine on cefpirome tissue concentration

Results of haemodynamic measurements

FBF measurements, MAP and HR were not significantly different between study days at baseline ($P > 0.05$). No significant changes versus baseline were detected for FBF measurements following NOR administration ($P > 0.05$; Figure 2). Systolic, diastolic and MAP increased significantly ($P = 0.017$) compared with baseline in the NOR group, only. Mean MAP increased from 73.3 ± 3.5 mmHg at baseline to 94.0 ± 5.2 mmHg during continuous NOR infusion and remained constant over 180 min ($P > 0.05$), whereas MAP did not change versus baseline in the PL group (Figure 3).

Discussion

Several preconditions must be fulfilled for an antibiotic to become therapeutically effective. Most importantly, the causative pathogen has to be susceptible to the antimicrobial agent and the drug concen-
tration at the target site must exceed its MIC. However, blood flow is the most important determinant that governs the distribution of antimicrobial agents within compartments. In particular, the capillary surface area to volume ratio is considered essential for plasma to tissue equilibration. The administration of NOR induces vasodistraction of capillaries by reducing vascular resistance and thereby permits evaluation of the effects of local changes in blood flow on drug tissue penetration in humans (unpublished data). In this experiment, we have demonstrated that local warming of a lower extremity results in a significant increase in blood flow and is paralleled by a significant increase in antimicrobial tissue concentration in comparison to the untreated extremity.

One important limitation has to be considered in the interpretation of our results. This study has a power to detect changes of blood flow of ~15% and therefore we cannot exclude that smaller changes might have occurred but remained undetected. However, we consider such small changes clinically irrelevant. In addition, we cannot completely rule out that NOR caused a simultaneous activation of α- and β-adrenoceptors exerting opposite effects neutralizing each other on the tissue level. However, this appears unlikely because cutaneous blood vessels almost exclusively express α-receptors. Smooth muscle cells of blood vessels that supply skeletal muscles have both, α- and β-receptors, but α-mediated vasoconstriction clearly predominates β-mediated vasodilatation, which is indicated by the increase in MAP in the NOR group.

It is of relevance to note that for systemically administered agents, it is difficult to discriminate between local and systemic compensatory haemodynamic mechanisms, which possibly alter changes in peripheral blood flow measured by plethysmography. This limitation might be overcome by use of methods that allow for local administration of vasoactive compounds and the simultaneous measurement of tissue concentration profiles of systemically administered antimicrobial agents at the site of drug action. The intra-arterial administration of NOR and microdialysis used as a drug delivery device permits evaluation of the effects of local changes in blood flow on drug distribution into tissues without systemic involvement.

In conclusion, we were able to demonstrate that penetration of cefpirome into soft tissues remained unaffected in healthy men, although significant changes in systemic haemodynamic parameters were observed during NOR administration. Most probably, this is because of compensatory mechanisms such as the decrease in heart rate and increase in vascular perfusion pressure, which maintain blood flow sufficiently high at peripheral sites in healthy volunteers.

**Acknowledgements**

This study was supported by the ‘Jubiläumsfonds der österreichischen Nationalbank’ Project number: 9355.
Effect of norepinephrine on cefpirome tissue concentration

References


