Penetration of Cefotetan into Suction Skin Blister Fluid and Tissue Homogenates in Patients Undergoing Abdominal Surgery

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The penetration of cefotetan (2-g intravenous bolus) into the suction blister fluid and tissue homogenates of 11 patients was investigated. Mean concentrations in tissue were significantly lower than contemporary suction blister fluid levels. These data show that the determination of beta-lactam concentrations by the tissue homogenate method may seriously underestimate the actual antibiotic levels in extracellular fluid.

The most important pharmacokinetic characteristic for an antimicrobial agent is adequate tissue distribution, i.e., the ability to achieve the concentrations necessary and sufficient for clinical effectiveness (8, 10, 11).

However, the problem of tissue distribution is often analyzed too simplistically, without consideration of the very different results obtained in relation to the various study methods, the different physicochemical drug parameters, the study population, and the type of result evaluation (1–4). The presence of blood in tissues, the degree of inflammation, the different sampling methods, and the antibiotic dosage (chemical, biological, etc.) are other factors that may interfere with the final concentrations (8, 9, 17).

The most widely used model is based on tissue homogenates collected during surgery. It is usually impossible to collect a series of specimens from the same subject, so final concentrations are the means from a group of subjects with considerable interindividual variations (4).

Drug extracellular concentrations have been studied with several models. Some of these use naturally produced fluids, such as pleural, peritoneal, synovial, and lymphatic fluids, whereas others use mechanisms that lead to inflammation fluid production (such as skin chambers, cantharidin blisters, and implanted cotton threads) or transudation (skin windows and suction blisters) (8).

The most widely used techniques are the suction and cantharidin blisters, which are easily used, acceptable to a patient, and reproducible.

In view of all the problems mentioned above, we studied the tissue penetration of cefotetan by comparing two methods, i.e., tissue homogenates and suction blisters, performed with patients undergoing abdominal surgery.

Eleven patients undergoing elective colorectal surgery, nine males and two females, with a mean age of 53.6 ± 15.9 years and a mean weight of 62.9 ± 12.2 kg, normal renal and hepatic functions, no history of allergy to beta-lactams, and no antibiotics during the previous week, entered the study. Four patients had Crohn’s disease, two had ulcercolitis, and five had colorectal cancer. Informed consent was obtained from all subjects.

Cefotetan (Zeneca S.p.A., Milan, Italy) was administered as a single 2-g intravenous (i.v.) bolus dose immediately (5 min) before the operation. Suction blisters of approximately 8 mm in diameter were induced on the volar surface of the forearm the day before (14 to 18 h before the operation) according to the method of Kiiastala as modified previously (12). Venous blood and suction blister fluid (SBF) were sampled before the administration of cefotetan and at 5 min (only blood) and 1, 2, 3, 6, 8, and 12 h after the drug was administered.

Tissue samples (skin, subcutaneous tissue, and colonic wall) were obtained from the same patients 1, 2, 3, and 6 h after injection. For surgical reasons, samples of skin or colonic wall were not taken from all 11 patients at the same times. Tissue and SBF samples were collected simultaneously at the first and second hours in two cases; at the first, second, and third hours in two cases; at the third hour in three cases; and at the sixth hour in four cases.

Tissues were rinsed with sterile normal saline to remove excess blood, dried, homogenized at 4°C with Polytron PT 10-35 homogenizer (Kinematica, Lucerne, Switzerland), and low-speed centrifuged, and the supernatant was used for the assay. All samples were stored at −80°C until assayed.

Standards were prepared daily in pooled human plasma for

FIG. 1. Mean (± standard deviation) observed concentration versus time curves (best fit computer lines) of cefotetan in plasma (solid circles) and SBF (open circles) samples from 11 patients after a single i.v. dose of 2 g.

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TABLE 1. Main pharmacokinetic parameters of cefotetan in plasma and SBF samples from 11 patients after a single i.v. 2-g bolus dose

| Type of sample | \( C_{\text{max}} \) (\( \mu \text{g/ml} \))\( ^{a} \) | \( T_{\text{max}} \) (h)\( ^{b} \) | \( t_{1/2} \) (h)\( ^{c} \) | AUC_{0-\infty} (\( \mu \text{g} \cdot \text{h/ml} \)) | \( V \) (liters)\( ^{d} \) | \( k_{\text{el}} \) (h\(^{-1}\))\( ^{e} \) | MRT (h) | PI (%) |
|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Plasma        | 212.9 ± 154.3   | 3.9 ± 0.9       | 3.3 ± 1.9       | 599.0 ± 100.1   | 18.7 ± 6.1      | 0.19 ± 0.4      | 5.1 ± 1.5       | 115.3 ± 41.0    |
| SBF           | 70.7 ± 22.7     | 4.8 ± 0.9       |                | 732.7 ± 235.6   |                | 0.15 ± 0.03     | 8.6 ± 0.7       |

\( ^{a} \) Data are means ± standard deviations.
\( ^{b} \) Computed value.
\( ^{c} \) \( T_{\text{max}} \) time to reach peak concentration.
\( ^{d} \) \( t_{1/2} \) elimination half-life.
\( ^{e} \) \( V \), volume of distribution at \( \beta \) phase.
\( ^{f} \) \( k_{\text{el}} \), elimination rate constant.

Blood samples, in 70% serum for SBF samples, and in normal saline for tissue specimens. Cefotetan concentrations were determined in triplicate by a standard large-plate agar diffusion technique with antibiotic medium 1 (Difco) (final pH 6.6), *Escherichia coli* SC 12155 as the test organism, and a lower limit of sensitivity of 0.5 \( \mu \text{g/ml} \) (7).

Best fit standard curves were obtained by linear regression analysis. The linearity between 0.5 and 8 \( \mu \text{g/liter} \) was log \( y = 0.113 x - 1.7 \) for plasma samples, log \( y = 0.111 x - 1.09 \) for SBF samples, and log \( y = 0.085 x - 1.12 \) for tissue samples with a correlation coefficient no less than 0.99. For all samples, intra-assay precision ranged from 4.7 to 7.5% and interassay precision at a level of 2 \( \mu \text{g/liter} \) ranged from 3.9 to 4.8%. Pharmacokinetic analysis of plasma and SBF concentrations was performed with a computerized program (Siphar, version 4.0; SIMED), using a two-phase model, on the basis of the extended least squares regression method with the Powell minimization algorithm. The area under the concentration-time curve and the mean residence time (MRT) were determined by trapezoidal rule and extrapolated to infinity, and the elimination half-life and volume of distribution at \( \beta \) phase were calculated by conventional methods (6). The penetration index (PI) was calculated as the ratio between the SBF and plasma areas under the concentration-time curve.

Figure 1 shows the concentration versus time curves of cefotetan. The mean peak concentration in plasma (\( C_{\text{max}} \)) was 213.0 ± 144.3 \( \mu \text{g/ml} \) and at 12 h, the minimum observed concentration was 11.3 ± 1.9 \( \mu \text{g/ml} \). The mean blister fluid \( C_{\text{max}} \) was 65.7 ± 30.1 \( \mu \text{g/ml} \) while its minimum observed concentration at 12 h was 34.4 ± 1.6 \( \mu \text{g/ml} \).

The main kinetic parameters are reported in Table 1. The mean computed \( C_{\text{max}} \) was 212.9 ± 154.3 and 70.7 ± 22.7 \( \mu \text{g/ml} \) for plasma and SBF samples, respectively, with a mean calculated time to reach peak in SBF samples of 3.3 ± 1.9 h. The elimination half-life was 3.9 ± 0.9 h for plasma samples and 4.8 ± 0.9 h for SBF samples with an MRT of 5.1 and 8.6 h, respectively, and similar values for areas under the concentration-time curve in both compartments. Therefore, the PI was 115.3 ± 41.0%, confirming a good diffusion of this cephalosporin in well-vascularized tissues (Table 1).

On the other hand, concentrations of cefotetan in plasma, SBF, and tissue (skin, subcutaneous tissue, and colonic wall) samples obtained simultaneously from the same patient during surgery in relation to sampling time after injection are shown in Table 2. Overall, mean concentrations in tissues were 2.5 to 5 times lower than the contemporary values observed with plasma samples. Although no significant difference between tissue values related to the same sampling times was observed, they were always significantly lower than the corresponding SBF levels.

Ryon and Cars first observed lower concentrations of ampicillin, cefoxime, and ceftazidime in muscle homogenates of rabbits than in muscle extravascular fluid obtained with implanted cotton threads or paper disks (14, 15).

In a study involving only two patients, Cars et al. found higher concentrations of piperacillin in wound fluid than in wound subcutaneous tissues (5). Our data with the suction blister method demonstrate that cefotetan half-life and MRT are higher in SBF than in plasma and that the minimum observed concentration is threefold more elevated (34.4 ± 1.6 \( \mu \text{g/ml} \) at 12 h).

On the other hand, concentrations in tissue homogenates are constantly and significantly lower than those in SBF, with values ranging from 24 to 49% of the corresponding blister fluid levels. Hydrophilic molecules, such as most of the beta-lactam antibiotics, are not uniformly distributed within tissue but remain in extravascular fluid, which amounts to only 10 to 30% of total tissue volume, as the rest is intracellular fluid (70 to 90%) or blood (3 to 6%) (4, 10). Thus, the observed

TABLE 2. Mean concentrations of cefotetan in plasma, SBF, skin and subcutaneous tissue, and colonic wall samples after a single i.v. 2-g bolus dose

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Mean concn (( \mu \text{g/ml} ) or ( \mu \text{g/g} )) at:</th>
<th>( P^{d} ) (SBF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h (( n = 4 ))</td>
<td>2 h (( n = 4 ))</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>105.2 ± 7.5</td>
<td>65.1 ± 22.1</td>
</tr>
<tr>
<td>SBF</td>
<td>48.2 ± 11.7</td>
<td>57.7 ± 11.4</td>
</tr>
<tr>
<td>Skin or subcutaneous</td>
<td>20.3 ± 3.1</td>
<td>18.8 ± 10.1</td>
</tr>
<tr>
<td>Colonic wall</td>
<td>ND(^{b})</td>
<td>ND(^{b})</td>
</tr>
</tbody>
</table>

\( ^{a} \) Data are means ± standard deviations.
\( ^{b} \) ND, not done.
\( ^{c} \) No significant difference was observed between skin or subcutaneous tissue and colonic wall values.
\( ^{d} \) One-way analysis of variance.
concentrations in tissue homogenates may be lower than the actual ones, since intracellular fluid may considerably dilute the specimen with a subsequent decrease in the total amount of measured antibiotic (13). On the contrary, beta-lactam distribution in the extravascular compartment provided by blister fluid is good and the achievement of steady state with the vascular compartment is fast. PI values range from 60 to over 90%, depending on the type of molecule and its physicochemical and pharmacokinetic properties (8).

These differences are confirmed by the fact that if we consider the influence of surface and volume on the kinetics of drugs in various tissue compartments, we can expect lower concentrations in models like SBF (relatively low surface-to-volume area) than in tissue homogenates (high surface-to-volume area) (16).

In conclusion, we can confirm from our data that the determination of beta-lactam antibiotic concentrations by the tissue homogenate method can seriously underestimate the actual antibiotic level in extracellular fluid, where the most common pathogens reside in cases of infection (4, 10, 11).

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REFERENCES


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