Sulfonamide and Trimethoprim Concentrations in Human Serum and Skin Blister Fluid

JOHAN N. BRUUN,† NILS ØSTBY,† JAN E. BREDESEN,‡ PETER KIERULF,# and PER KNUT M. LUNDE‡

Medical Department † and The Central Laboratory, ‡ Ulleval Hospital, Oslo, Norway

Various sulfonamides and trimethoprim were given orally twice a day to healthy volunteers. The drug concentrations in serum and tissue fluid from skin blisters were determined concomitantly. Maximal serum concentrations were obtained after 1 to 3 h. Absorption of sulfacarbamide and sulfadimidine was more rapid than for sulfadiazine, sulfamethoxazole, and trimethoprim. The penetration to blister fluid was delayed and maximal concentrations were usually reached after 4 to 8 h. The highest penetration to blister fluid was found for sulfacarbamide, sulfadiazine, and trimethoprim. During maintenance therapy sulfadiazine and trimethoprim gave blister fluid concentrations usually above 50% of the serum level. However, on the basis of dosage the highest sulfonamide concentration both in serum and blister fluid was obtained with sulfamethoxazole.

The effect of treatment with antibacterial drugs depends on the penetration of the drug to the focus of infection. The dosage of the drugs is usually based on knowledge of concentrations achieved in serum or plasma. However, most infections are located outside the blood stream, and determination of the concentration obtained in the actual focus would be more appropriate.

Suction blisters of the skin have been used to study the penetration of, e.g., penicillins to tissue fluid (7, 8, 12). Antibiotic penetration to tissue fluid has also been studied using tissue cages and skin abrasion techniques (1, 2, 5, 13, 14). The penetration of the drugs to tissue cages and skin blisters is slower than to the fluid sampled with the skin abrasion techniques (5, 7). The fluid of skin blisters has, therefore, been regarded as a "deeper compartment" than the fluid of the interstitial space (7). Since many foci of infection also may be regarded as "deep compartments," the study of penetration of antibacterial agents to skin blisters is thought to be of considerable interest.

MATERIALS AND METHODS

Volunteers. Ten healthy medical students, six males and four females, from 22 to 27 years of age and weighing from 56 to 78 kg, participated in the study. They had all given their informed consent, and examination of serum creatinine, hemoglobin, electron spin resonance, and urine (biochemical and microscopy) showed normal values.

Skin blisters. Suction blisters were produced by the method described by Hellum and Solberg (9). A Perspex block with eight bores was strapped to the volar area of the forearm after the skin had been washed with 70% alcohol. Suction at a negative pressure of 0.3 kg/cm² was applied until half-spherical blisters had been produced. This usually took 1.5 to 2 h. Skin blisters were made on day 1 on one arm and on day 4 on the other arm. Blister production was completed within the last 30 min before the drugs were given.

Medication. The volunteers were divided into two groups of five, and each was given one of the following two preparations: (i) tablets containing trimethoprim at 80 mg and sulfamethoxazole at 400 mg (Bactrim; Roche); or (ii) tablets containing sulfacarbamide at 167 mg, sulfadiazine at 167 mg, and sulfadimidine at 167 mg (Trisulfamid; Weifa). On day 1, immediately after the suction blisters had been made, the volunteers received four tablets of one of the above-mentioned preparations on an empty stomach. Subsequently they were given two tablets every 12 h for 4 days.

Sampling. Serum samples were obtained by venipuncture. Tissue fluid samples were obtained by puncture and aspiration of 0.10 to 0.15 µl of fluid from skin blisters made the same day. Serum and skin blister fluid samples were obtained on days 1 and 4 before and 1, 2, 3, 4, 8, and 12 h after the morning dose of the drug had been given.

Protein assay. The total protein content of blister fluid was determined essentially by the method of GoA (6), using a biuret reaction; the spectrophotometric readings were performed at 330 nm. Before precipitation with trichloroacetic acid (110 g/liter), the samples were diluted 30- to 40-fold with 0.15 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) to obtain appropriate sample volumes. Samples thus diluted were precipitated with 4 volumes of trichloroacetic acid.

Drug assay. The concentration in serum and tissue fluid of trimethoprim and the parent (active) sulfonamide compounds was determined by using liquid chromatography (15). The readings were performed at 254 nm with a Spectra Physics 3000 B high-performance liquid chromatograph equipped with a model 8300 spectrophotometric detector.
RESULTS

The total protein content determined in blister fluid from seven subjects was 29.9 ± 6.7 g/liter (mean ± standard deviation). There was a mean increase of 24% in protein content from the first to the last sample each day.

The mean total serum concentrations at different intervals after the drug was given are shown in Fig. 1. For sulfacarbamide and sulfadimidine, maximum serum concentrations were found 1 to 2 h after ingestion of the drug, whereas the other agents gave peak concentrations after 2 to 3 h.

Figure 2 shows the mean concentrations in tissue fluid from the skin blisters. The penetration to the blister fluid was delayed for all agents, and the maximum level was apparently reached 4 to 8 h after the drug was given.

On day 4 the concentrations of sulfadiazine and trimethoprim in blister fluid were usually above 50% of the serum level, whereas the other agents often gave values less than 40% of that found in serum. The highest sulfonamide levels were obtained with sulfamethoxazole. Even if this was corrected for the higher dosage, the concentrations both in tissue fluid and serum were relatively higher than for the other sulfonamides.

Table 1 gives the ratio between simultaneous blister fluid and serum concentrations, and Table 2 gives the ratio between the peak blister fluid and the peak serum concentration. The ratios on day 4 are probably the most appropriate reflection of the penetration of the drugs during maintenance therapy. Higher ratios were obtained for sulfacarbamide, sulfadiazine, and trimethoprim than for the other agents.

DISCUSSION

Examination of drug penetration to tissue fluid from tissue cages requires surgical intervention. Such studies have mainly been adopted in animals (2), although some studies have also
been performed in humans (1). However, the skin blister technique does not require surgical intervention and is well tolerated. The skin window technique used by Tan and Salstrom (13, 14) measures penetration into chambers filled with saline which is changed between each sample. The present method measures the drug penetration into blisters filled with the subject's own interstitial fluid. Accordingly, the skin blister is presumably more relevant to the clinical situation.

The results from penetration studies in animals are not directly applicable to humans. Thus, Piercy (11) observed differences between animal species in the diffusion of sulfadiazine to tissue fluid.

The penetration of drugs to tissue fluid has been expressed in different ways. Tan and Salstrom (13, 14) used the ratio between mean tissue fluid and serum concentrations obtained simultaneously. Presumably the mean of such ratios from individual patients would be more appropriate. As seen from our results (Table 1) based upon successive sampling from separate blisters prepared before the dose was given, the ratio will exceed 1 with dosage intervals of some length. Besides dosage interval and the time of sampling, the ratio measured may depend on whether a steady state is reached, as well as the protein content and drug binding capacity of the blister fluid as compared to serum. Accordingly, the peak concentration ratio between tissue fluid and serum (Table 2) would be the most relevant indicator for the degree of drug penetration to the tissues. The highest ratios on both days 1 and 4 were found for trimethoprim, sulfacarbamide, and sulfadiazine. The highest blister fluid concentration for these drugs varied between 60 and 90% of the serum level, suggesting a better penetration of these drugs than for the other drugs included in the study.

The penetration of antimicrobial agents to tissue fluid has been found by some authors to be inversely related to the degree of plasma protein binding (1, 2, 14). Others (5) have not been able to confirm this. In the present study (Tables 1 and 2) the lowest blister fluid/serum concentration ratios were seen with sulfadimidine and sulfamethoxazole, which are the two most extensively bound sulfonamides of the four tested (10). However, as already mentioned, the protein content in the tissue (or blister) fluid must also be considered, including the qualitative aspects. Thus albumin seems to be the most important carrier protein for acidic drugs such as sulfonamides, whereas other protein fractions such as orosomucoid (3) seem to be more dominant with regard to binding of basic drugs, presumably including trimethoprim. Accordingly, when trying to explain the distribution of antimicrobial and other drugs to tissue fluids, the relative concentrations of relevant protein

### Table 1. Ratios between blister fluid concentrations and serum concentrations of sulfonamides and trimethoprim

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>8</th>
<th>12</th>
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<tbody>
<tr>
<td>Sulfacarbamide</td>
<td>1</td>
<td>0.36</td>
<td>0.55</td>
<td>1.07</td>
<td>1.20</td>
<td></td>
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<td></td>
<td>4</td>
<td></td>
<td></td>
<td>0.56</td>
<td>1.13</td>
<td>1.06</td>
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<td></td>
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<tr>
<td>Sulfadimidine</td>
<td>1</td>
<td>0.16</td>
<td>0.26</td>
<td>0.48</td>
<td>0.87</td>
<td>0.80</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td>0.22</td>
<td>0.41</td>
<td>0.43</td>
<td>0.71</td>
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<td>Sulfadiazine</td>
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<td>0.19</td>
<td>0.32</td>
<td>0.58</td>
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<tr>
<td></td>
<td>4</td>
<td>0.47</td>
<td>0.65</td>
<td>0.60</td>
<td>0.77</td>
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<td>Sulfamethoxazole</td>
<td>1</td>
<td>0.12</td>
<td>0.34</td>
<td>0.36</td>
<td>0.59</td>
<td>0.68</td>
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</tr>
<tr>
<td></td>
<td>4</td>
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<td>0.41</td>
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<td>0.83</td>
<td>0.73</td>
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<tr>
<td>Trimethoprim</td>
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<td>0.35</td>
<td>0.64</td>
<td>0.59</td>
<td>0.76</td>
<td>0.80</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.66</td>
<td>0.61</td>
<td>0.89</td>
<td>1.06</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of ratios from five subjects. — Mean ratio not calculated because of too low concentrations in the sample from two or more subjects.

### Table 2. Ratio between highest blister fluid and serum levels

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mean ratio ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Sulfacarbamide</td>
<td>0.62 ± 0.05</td>
</tr>
<tr>
<td>Sulfadimidine</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0.61 ± 0.13</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>0.48 ± 0.05</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.66 ± 0.07</td>
</tr>
</tbody>
</table>

*Mean of ratios from five subjects.
fractions should be considered, as well as the consequences of the physicochemical characteristics of the drugs (pKₐ, lipid solubility) in relation to the local circulatory and acid-base balance situation.

In this study various commercial fixed drug combinations were given in recommended doses. The simultaneous administration of two or three drugs might reduce the variability in pharmacokinetics due to intra- as well as inter-individual differences in physiological and pathophysiological functions. However, the pharmacokinetics of one drug may also be influenced by other drugs. Thus, the pharmacokinetics of each of the drugs studied may be somewhat different if the drugs are given separately. Previous studies suggest that the problem of altered pharmacokinetics when fixed combinations of sulfonamides are given is quite limited (4).

LITERATURE CITED