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Effect of Protein Binding on Drug Penetration into Blister Fluid

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The effect of protein binding on drug penetration into blister fluid was evaluated by using cefonicid, ceftizoxime, and cefotaxime. Drug concentrations in a chamber with a high surface area/volume ratio (i.e., paper disk) follow changes in serum more closely than do those in a chamber with a low surface area/volume ratio. Both the area under the concentration-time curve ratio and the concentration ratio (by the disk method) for cefonicid were statistically lower than the ratios for ceftizoxime and cefotaxime. The high degree of protein binding of cefonicid results in the availability of less drug for diffusion to blister fluid than with the low-protein-binding ceftizoxime and cefotaxime.

Drug concentrations in blister fluid are critical, since most bacterial infections occur in the interstitial space and not the intravascular space. Many investigators have tried to correlate the penetration of β-lactam antibiotics into interstitial fluid with the degree to which the drugs bind protein. However, such studies have yielded disparate results, as shown by reports of poor cefazolin extravascular penetration in one study (11, 12) and of good penetration in other studies (1, 4, 5, 7, 8).

Cefonicid is 98% bound to serum protein and is used for the treatment of skin and soft tissue infections (3). In view of the high degree of protein binding of this compound, the penetration of the drug into blister fluid was compared with the penetrations of ceftizoxime and cefotaxime (<40% bound) by using a modified suction blister technique (10).

Six healthy volunteers (four male and two female) between the ages of 25 and 29 years and weighing 51 to 80 kg were enrolled in this study. Female volunteers were not pregnant or receiving oral contraceptives.

Each subject received a single 30-mg/kg (body weight) dose of cefonicid, ceftizoxime, or cefotaxime on three occasions. A treatment schedule was assigned randomly to each subject. There was at least 1 week between doses. Drugs were given by constant intravenous infusion over 5 min into an antecubital vein via an indwelling intravenous catheter. Normal saline (3 ml) was injected immediately after the infusion to flush the catheter. Blood samples were obtained from the cannulus, after discarding the first 2 ml, at 0, 5, 10, 20, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, 600, 720, and 1,440 min after dosing. Each volunteer had eight skin blisters produced on his or her forearm by the modified suction blister technique previously described by Shyu et al. (10).

In the protein-binding study, cefonicid standards were prepared in freshly pooled human serum at 37°C at 450, 400, 350, 300, 250, 175, 150, 125, 100, 75, 50, 25, and 12.5 μg/ml. A portion (1 ml) of the serum sample was transferred into an Amicon MSP-1 ultrafiltration unit with YMT membranes immediately after preparation. Ultrafiltration was performed at 37°C and 1,000 × *g* for 15 min.

Cefonicid concentrations were determined by a microbiological assay with *Bacillus subtilis* ATCC 6633 as the test organism. Serum standards were prepared in pooled serum, and blister fluid standards were prepared in 50% pooled serum and 50% phosphate buffer (0.1 M, pH 7.4). Protein-free filtrate standards were prepared in phosphate buffer (0.1 M, pH 7.4), which was found to produce the same detection response as protein-free filtrate (data not shown). The assay limits for cefonicid were 12.5, 6.3, and 0.5 μg/ml in serum, blister fluid, and protein-free filtrate samples, respectively. The assay coefficients of variation between days and within day were less than 10%.

The area under the concentration-time curve (AUC) ratio of drug in blister fluid from each subject was calculated as follows: AUC ratio = $AUC_{blister}/AUC_{serum} \times 100$, where $AUC_{blister}$ and AUC_{serum} represent the AUCs of blister fluid and serum, respectively. Both AUC_{serum} and $AUC_{blister}$ were calculated by the linear trapezoidal rule and estimated the last concentration in serum to infinity by serum concentration/slope of the elimination phase.

The AUC ratio is a good indicator of the total amount of drug that reaches an extravascular compartment. To avoid comparative error in AUC ratio calculations, there must be enough concentration measurements to characterize the drug concentration-time profiles. For this reason, the AUC ratio could not be calculated for cefonicid when the blister technique was used.

The concentration ratio of drug in blister fluid was calculated as follows: concentration ratio = (blister concentration/serum concentration) × 100, where blister concentration and serum concentration are the simultaneous drug concentration in blister fluid and serum, respectively. This measurement is a valid estimation of penetration into an extravascular compartment only after distribution to various body sites is accomplished, i.e., during the elimination phase. The measurement is also inaccurate if the time lapse to the elimination phase is prolonged by artifacts in the sampling method (e.g., skin blister fluid). There are difficulties in determining when the purely elimination phase begins. For these reasons, this calculation can be applied only to the results obtained by the paper disk method.

In the concentration range below 175 μg/ml, the fraction of

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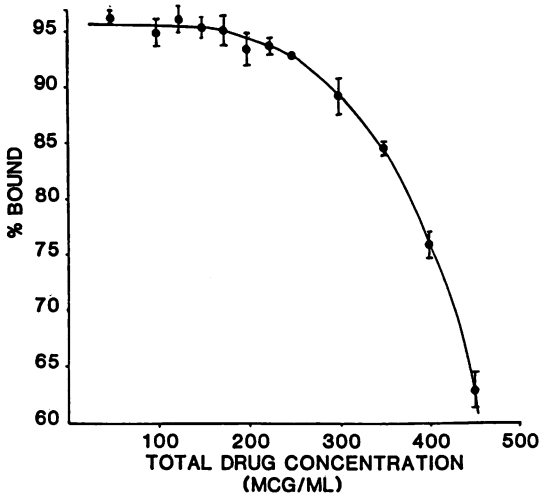

FIG. 1. Percentage of bound cefonicid versus total drug concentration.

cefonicid bound was 96.0 ± 0.4%. Pronounced nonlinear binding of cefonicid to serum protein occurred as concentrations exceeded 175 μg/ml (Fig. 1). Scatchard plot analysis of the binding data suggested only one class of binding site. The number of binding sites and binding affinity constant obtained from this study, assuming the binding protein is albumin, were 0.95 ± 0.14 and (6.3 ± 2.8) × 10^4/M, respectively.

By the paper disk method, the cefonicid blister pharmacokinetic profile was similar to the serum pharmacokinetic profile (Fig. 2A). The cefonicid concentration in blister fluid at 30 min was 44 ± 15 μg/ml. The elimination half-lives in blister fluid and serum were 4.4 ± 1.0 and 4.1 ± 0.8 h, respectively. The AUC and concentration ratios were 23.2 ± 1.1 and 26.3 ± 0.9%, respectively.

The protein concentration in blister fluid collected before administration of the drug and during the experimental period was 3.9 ± 0.4 g/dl (n = 32). The distribution of cefonicid into suction blisters was delayed, with peak concentrations occurring 5 h postdose (Fig. 2B). The peak concentration of cefonicid in the blister fluid was 47 ± 10 μg/ml. The elimination half-lives in blister fluid could not be determined, since an elimination phase could not be identified.

The data for ceftizoxime and cefotaxime were previously published as part of a methodological study (10).

Previous studies have noted the importance of the surface area/volume (SA/V) ratio of an extravascular compartment when the pharmacokinetics of drugs in tissue fluid are evaluated (9, 10, 13). As observed in previous studies, drug concentrations in fluid spaces with a high SA/V ratio (i.e., paper disk) tend to more closely follow changes in the intravascular space, whereas drug concentrations in spaces with a low SA/V ratio (i.e., blister fluid) equilibrate more slowly, i.e., exhibit delayed and depressed peak concentrations (Fig. 2B).

Our data demonstrate that cefonicid is highly bound to serum proteins. Binding also significantly influences the pharmacokinetics of this drug in blister fluid, since the fluid contains a high protein concentration (approximately 50% of that found in serum). This resulted in a delayed peak drug concentration in blister fluid (approximately 5 h postdose) for cefonicid compared with the peaks observed for ceftizoxime and cefotaxime, which are less than 40% bound to plasma proteins (2) (approximately 1 to 1.5 h after the dose; 10). This was consistent with the results of previous studies which compared drugs with high and low degrees of binding to serum proteins (6, 9, 14). The crossover of serum and blister fluid curves with the blister technique presents difficulties in accurately determining the penetration of a drug, regardless of the degree of protein binding. It is suggested that those parameters not be calculated from experiments which use collection devices with low SA/V ratios.

When a device with a high SA/V ratio, i.e., a paper disk, was used, a parallel between total cefonicid pharmacokinetics in serum and blister fluid was observed (Fig. 2A). Ceftizoxime and cefotaxime also demonstrated the same characteristics (10). The peak blister concentrations for all three compounds were reached within 30 min (the earliest sampling time), indicating that penetration into blister fluid was rapid. The drug concentration ratios of cefonicid, ceftizoxime, and cefotaxime were 26.3, 35.1, and 30.5, respectively, in simultaneously drawn interstitial fluid and serum samples (statistically significant, $P < 0.05$ by the Student t test). It appears that the high degree of protein binding of cefonicid decreases its availability to blister fluid and presumably to interstitial fluid.

In summary, these data demonstrate that despite high

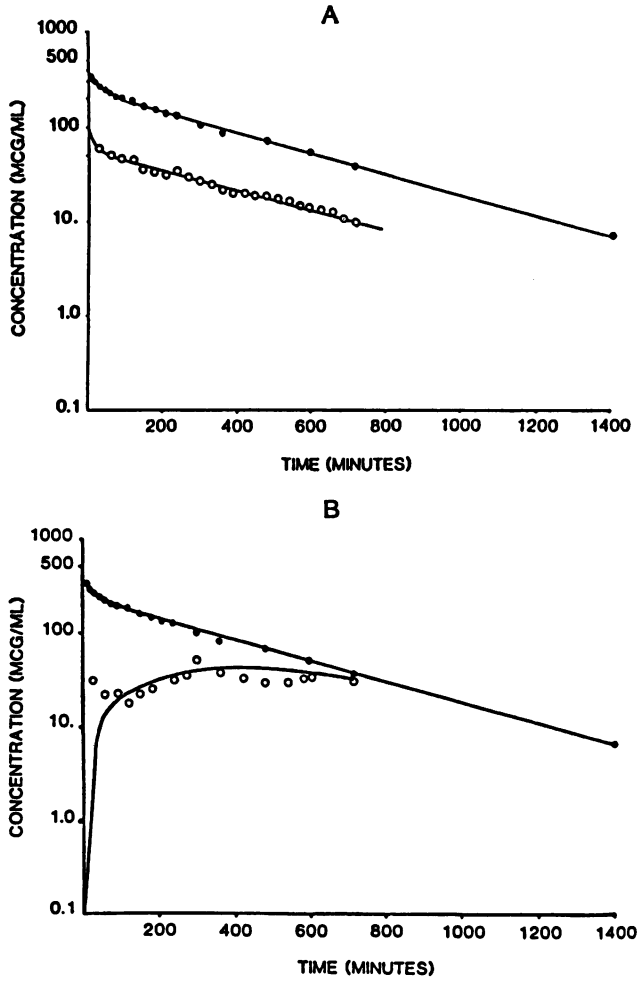

FIG. 2. Concentration-time curves for cefonicid in serum (●) and in blister fluid (○) after a single 30-mg/kg intravenous bolus dose in a representative volunteer. Blister fluid was obtained by the paper disk (A) and blister fluid (B) methods.

binding to serum proteins, total cefonicid concentrations in blister fluid are inhibitory for many human pathogens (3). Compared with distribution of drugs with low degrees of plasma protein binding, such as ceftizoxime and cefotaxime, the distribution of cefonicid into a fluid with a small SA/V ratio, e.g. skin blisters, was delayed. Despite nonlinear serum protein binding after a single dose, this was particularly true in protein-containing fluid spaces with small SA/V ratios. A high degree of protein binding also made less drug available to the interstitial fluid than was available with similar compounds with low-protein-binding properties. This is probably not a problem when the target microorganism is extremely susceptible to cefonicid but may be a factor in microbial eradication for moderately susceptible organisms.

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