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Influence of Serum Protein Binding and Mode of Administration on Penetration of Five Cephalosporins into Subcutaneous Tissue Fluid in Humans

BJÖRN HOFFSTEDT[1]* AND MATS WALDER[2]

Department of Infectious Diseases[1] and Department of Clinical Bacteriology,[2] University of Lund, Malmö General Hospital, S-214 01 Malmö, Sweden

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The penetration of five cephalosporins into interstitial fluid was investigated by a new method employing cotton threads implanted subcutaneously. Penetration after short bolus injections, calculated as area under interstitial fluid level curve divided by area under serum level curve × 100, was 47.0% for cephradine, 30.6% for cefuroxime, 26.7% for cefotaxime, 24.6% for cefoxitin, and 9.6% for cefoperazone. There was an inverse correlation between the degree of penetration and serum protein binding with $r = -0.97$. The area under interstitial fluid level curves was the same whether the drugs were administered as short bolus injections or short time infusions.

It is generally maintained that only the free, unbound drug is available for antibacterial activity and for distribution to the extravascular compartments. Therefore, for treatment of infections outside the vascular compartment, drugs which are minimally bound to serum proteins are regarded as superior to drugs which are highly bound to the serum proteins (11, 14, 18). Although a number of models have been established to evaluate the influence of serum protein binding on antibiotic pharmacokinetics, especially in animals (4, 12, 17, 18, 20), all conclusions are far from certain (15).

It is likewise unclear what role the rates of drug delivery play in the penetration of the agent into the extravascular compartment. In one model an infusion for 15 min was preferential over a 1-h infusion or a bolus injection because it yielded higher levels in subcutaneous cage fluid in rabbits (5). In another model the high peak was regarded as important for penetration into extravascular foci (3). In an effort to clarify these issues, we investigated the penetration into the interstitial fluid (IF) of five cephalosporins in humans.

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MATERIALS AND METHODS

The antimicrobial agents used were cephradine (13), cefuroxime (7), cefotaxime (6), cefoxitin (8), and cefoperazone (19), of which the serum-protein-binding capacities were 10, 33, 40, 70, and 90%, respectively. The serum-protein-binding capacities varied over the concentration range of the drugs, but the figures mentioned are average values over the normal serum concentration range.

The investigation was approved by the Ethical Committee of the University of Lund, and the volunteers were verbally informed and gave their verbal consent. Three healthy male and four healthy female volunteers, with no known allergy to penicillin and no history of antimicrobial therapy during the previous month, were treated with each drug. Their mean age was 32.6 years ± 7.3 (standard deviation), and their mean weight was 70.4 kg ± 9.3 (standard deviation). All had normal serum creatinines.

Each subject received one intravenous injection for 5 min and after at least 1 week an intravenous infusion for 30 min. The doses were 1 g of cephradine, 1.5 g of cefuroxime, and 2 g of cefotaxime, cefoxitin, and cefoperazone.

The model used for collecting subcutaneous IF was that described by Ryan (16) and modified by Hoffstedt and Walder (10). After disinfection of the skin with an ethanol swab and administration of a local anaesthetic (1 to 2 ml of Xylocain, 10 mg/ml), two 22-cm-long cotton threads (umbilical tape Ethicon) were inserted subcutaneously for 1 to 2 cm. At different sampling times the threads were drawn through the subcutaneous tissue, cut off at positions previously placed subcutaneously, immediately placed in a plastic tube with a volume of 300 μl, and stored at −70°C until assayed. Six to seven samples could be obtained from each thread.

Only thread samples without macroscopically visible blood were analyzed.

Thirty minutes after the implantation of the threads (allowing the edema caused by the anesthetic to disappear), drugs were administered in the opposite arm. Samples from blood and threads were taken at 15, 60,

120, 240, 300, and 360 min after drug delivery.

Thread samples were analyzed by a microbiological method. Disposable plates (24 by 24 cm) containing 125 ml of DST Oxoid CM 261 agar with pH adjusted to 7.4 were used, with the exception of cefotaxime, where the agar was PDM-ASM agar (Biodisk). Wells with a diameter of 8 mm were made by a regular hole punch machine. Then 1 cm of thread was cut into smaller pieces and spread out in the well. The antibiotic was eluted by adding 100 μl of phosphate-buffered saline to each thread-containing well. Thread antibiotic standards were made by impregnating 1 cm of sterilized thread with different concentrations of antibiotic solutions.

Micrococcus luteus ATCC 9341 was the indicator strain for measurement of the concentrations of cephradine, cefuroxime, cefoxitin, and cefoperazone; *Escherichia coli* V6311/65 was the indicator strain for cefotaxime. This latter bacterium is susceptible to the desacetylmetabolite of cefotaxime. However, this metabolite has only 20% of the antibacterial activity of the parent drug and, as the degree of metabolization of cefotaxime was about 30%, the influence of this metabolite upon the obtained results was negligible.

The limit of sensitivity for the assay methods was 0.5 μg/ml for all drugs except cephradine and cefotaxime, where the limit was 0.25 μg/ml. The coefficient of variation (standard deviation as percentage of the mean concentration) varied for these assays from 8 to 13%. This coefficient of variation was in the same range for the serum assays.

The penetration, i.e., the relation between the area under the IF curve (AUC_{IF}) and the area under the serum curve (AUC_s) in percent, and the relation between peak IF level and peak serum level in percent for the tested drugs were calculated. The AUC was determined by the trapezoidal rule. The half-lives of the tested drugs in serum and IF and the duration of IF levels above 2 μg/ml were also calculated. The correlation between penetration and serum protein binding was determined from data on bolus injections and infusions.

Statistical methods. Statistical analyses were performed by using a paired *t* test on differences of values of AUC_{IF} and AUC_s.

RESULTS

The data in Table 1 show that the mode of administration had only a small influence on the levels of the test drug in serum but had a marked influence on concentrations in IF. The IF peak levels were delayed when the drugs were administered as short-time infusions. After 1 h the concentration curves in IF after injection and infusion were almost parallel.

After a bolus injection the penetration was 47.0% for cephradine, 30.6% for cefuroxime, 26.7% for cefotaxime, 24.6% for cefoxitin, and 9.6% for cefoperazone. Penetration after short-time infusions was almost identical with the exception of the most highly protein-bound drugs, cefoxitin and cefoperazone, which yielded somewhat higher figures (Table 2).

The elimination half-lives of cephradine, cefotaxime, and cefoxitin were equal in serum and IF, and no influence of mode of administration could be seen. Cefuroxime and especially cefoperazone yielded a prolonged half-life in IF compared to serum. This prolonged half-life in IF also influenced the duration of IF levels above 2 μg/ml. This time was about 4 h for cefuroxime and 5 h for cefoperazone compared to 3 h for the other drugs (Table 2).

There was no statistically significant differ-

TABLE 1. *Concentrations of cephradine, cefuroxime, cefotaxime, cefoxitin, and cefoperazone in serum (S) and IF*

Drug	Dose (g) and method of delivery[a]	Body fluid	Concn levels (μg/ml) ± standard deviation at (h):					
			0.25	1	2	4	5	6
Cephradine	1.0 s.b.	S	32.3 ± 10.6	11.5 ± 3.1	5.8 ± 1.8	1.8 ± 0.4	1.0 ± 0.3	0.5 ± 0.4
		IF	13.2 ± 5.7	9.4 ± 4.4	3.5 ± 1.3	1.7 ± 0.8	1.2 ± 0.6	0.7 ± 0.3
	1.0 inf.	S	21.5 ± 7.3	24.0 ± 8.1	5.1 ± 2.1	1.5 ± 0.3	0.8 ± 0.4	<0.25
		IF	1.5 ± 0.7	7.0 ± 3.2	2.1 ± 0.9	1.1 ± 0.5	0.6 ± 0.2	<0.25
Cefuroxime	1.5 s.b.	S	94.1 ± 8.2	45.1 ± 6.4	15.1 ± 3.4	3.6 ± 0.9	1.6 ± 0.3	0.7 ± 0.1
		IF	9.2 ± 3.7	11.8 ± 3.9	6.4 ± 2.1	2.0 ± 0.8	1.1 ± 0.6	0.7 ± 0.1
	1.5 inf.	S	91.3 ± 12.1	54.0 ± 8.9	20.3 ± 5.1	6.3 ± 1.6	2.9 ± 1.1	1.5 ± 0.6
		IF	2.1 ± 0.8	15.4 ± 4.1	7.0 ± 2.2	1.8 ± 0.6	1.1 ± 0.7	0.7 ± 0.1
Cefotaxime	2.0 s.b.	S	122.3 ± 11.1	50.1 ± 13.1	20.5 ± 4.9	4.7 ± 1.2	2.3 ± 0.6	1.0 ± 0.2
		IF	16.5 ± 2.4	12.7 ± 3.6	5.1 ± 2.8	0.8 ± 0.2	0.3 ± 0.2	<0.25
	2.0 inf.	S	109.2 ± 22.6	56.3 ± 12.7	15.9 ± 4.2	3.6 ± 0.9	1.8 ± 0.3	0.8 ± 0.1
		IF	7.1 ± 2.1	16.8 ± 0.3	7.4 ± 2.1	1.3 ± 0.4	0.3 ± 0.1	<0.25
Cefoxitin	2.0 s.b.	S	93.4 ± 9.7	31.2 ± 5.6	13.9 ± 3.1	1.9 ± 0.4	0.7 ± 0.2	<0.5
		IF	20.4 ± 3.7	17.1 ± 3.8	6.8 ± 1.5	0.9 ± 0.2	<0.5	<0.5
	2.0 inf.	S	76.4 ± 12.3	44.7 ± 8.7	12.4 ± 2.8	2.8 ± 0.9	1.3 ± 0.4	0.6 ± 0.1
		IF	14.2 ± 2.9	15.5 ± 2.9	5.0 ± 2.1	0.5 ± 0.1	<0.5	<0.5
Cefoperazone	2.0 s.b.	S	293.1 ± 42.4	104.1 ± 21.4	44.2 ± 7.9	21.0 ± 4.3	13.7 ± 2.9	8.6 ± 1.5
		IF	5.6 ± 1.1	8.0 ± 2.3	6.1 ± 1.4	3.4 ± 0.8	2.3 ± 0.6	1.7 ± 0.3
	2.0 inf.	S	182.3 ± 31.9	105.6 ± 29.1	49.9 ± 9.2	13.8 ± 4.1	7.8 ± 1.6	4.4 ± 0.9
		IF	1.2 ± 0.1	8.3 ± 1.9	6.2 ± 1.7	3.0 ± 1.0	2.1 ± 0.8	1.5 ± 0.4

[a] Abbreviations: s.b., short bolus injection; inf., infusion.

TABLE 2. *Pharmacokinetics of cephradine, cefuroxime, cefotaxime, cefoxitin, and cefoperazone*

Antibiotic	Serum protein binding (%)	Dose (g)[a]	$t_{1/2s}$ (min)	$t_{1/2IF}$ (min)	Time in IF above 2 μg/ml (h)	AUC_{IF} (μg·h/ml)	AUC_s (μg·h/ml)	$\frac{AUC_{IF}}{AUC_s}$ (%)	$\frac{Peak_{IF}}{Peak_s}$ (%)
Cephradine	10	1.0 s.b.	61	55	3.5	20.4 ± 6.9	43.4 ± 14.7	47.0 ± 0.1	41
		1.0 inf.	54	50	3.0	18.8 ± 9.5	36.5 ± 7.0	51.5 ± 12.5	23
Cefuroxime	33	1.5 s.b.	52	70	4.0	36.3 ± 7.0	118.6 ± 22.8	30.6 ± 8.3	14
		1.5 inf.	51	82	3.7	25.7 ± 1.0	93.4 ± 19.7	27.5 ± 4.6	20
Cefotaxime	40	2.0 s.b.	50	45	3.0	40.8 ± 18.1	152.7 ± 20.4	26.7 ± 4.2	14
		2.0 inf.	50	45	3.5	39.8 ± 3.7	131.1 ± 24.1	30.4 ± 8.1	20
Cefoxitin	70	2.0 s.b.	51	50	3.2	18.6 ± 7.6	75.6 ± 14.5	24.6 ± 4.5	22
		2.0 inf.	54	54	3.0	24.0 ± 17.3	56.9 ± 19.7	42.2 ± 10.9	26
Cefoperazone	90	2.0 s.b.	96	135	5.5	28.8 ± 3.0	299.9 ± 34.7	9.6 ± 1.3	5
		2.0 inf.	80	150	5.2	31.7 ± 3.7	215.1 ± 51.4	14.7 ± 6.1	6

[a] Abbreviations: s.b., short bolus injection; inf., infusion; $t_{1/2s}$ and $t_{1/2IF}$, half-lives of drugs in serum and IF, respectively.

ence in the AUC_{IF} when the drugs were given as bolus injections compared to short-time infusions.

The AUC_s varied between 299.9 μg.ml/h (cefoperazone) and 36.5 μg.ml/h (cephradine), and the AUC_{IF} varied between 40.8 μg.ml/h (cefotaxime) and 18.6 μg.ml/h (cefoxitin) (Table 2).

The relationships between peak levels in serum and IF varied between 41% (cephradine) and 5% (cefoperazone) (Table 2).

The correlation coefficient between the degree of penetration measured as described above and serum protein binding was $r = -0.97$. The correlation coefficient between AUC_{IF}/dose and serum protein binding was $r = -0.67$.

DISCUSSION

In humans Tan et al. found a great influence of serum protein binding on penetration of antibiotics into extracellular fluid (18). Wise et al. demonstrated a linked relationship between the degree of protein binding and the penetration of antibiotics (21).

In our study we used a "physiological" model with a low degree of inflammatory reaction and tissue destruction. This was indicated by the low total protein content (10). We were able to confirm the earlier results of Kunin et al. that only the free, unbound fraction of the antibiotic penetrates into the IF (11). As the AUC_{IF}/dose were almost identical for the low-protein-bound drugs and nearly twice that of the high-protein-bound cefoxitin and cefoperazone, this indicates the necessity of administering high doses of highly serum-protein-bound drugs to achieve therapeutic levels in IF. Conversely, low-serum-protein-bound drugs might achieve high IF levels with small doses.

The absolute levels of antibiotics found in the IF during our study were lower than those of Gillet and Wise (8). This can be explained by the fact that we had very little inflammatory reaction and tissue damage. Our levels and degree of penetration were also below those reported by Ryan, who used a technique similar to ours (16). However, there was a major difference in that Ryan implanted the threads 24 h before the antibiotic was administered, and hence the volunteer developed an inflammatory reaction around the threads.

The shape of the IF curves closely followed the serum curves, indicating that only passive diffusion moves the drug to and from the extravascular compartment. Cefoperazone showed a prolonged half-life in IF compared to serum. This may be due to its high protein-binding capacity and resultant binding to IF proteins creating a "depot"-like effect that can also be seen for other highly protein-bound drugs, such as doxycycline (1, 9). The lipophilic effect of the long side chain in position 7 of cefoperazone could also be responsible for this depot-like effect.

The influence of the mode of administration on the antibiotic levels in IF is still under debate. Carbon et al. found significantly higher IF levels after 15 min of infusion than after 1 h of infusion or bolus injection in rabbits (5). However, these values were achieved after two or three injections, and a cumulative effect was noted in IF for all modes of administration. Auvergnat, on the other hand, found no differences in cerebrospinal fluid levels in dogs of penicillin, ampicillin, and amoxycillin after several hours of therapy (2). Barza et al. found that in fibrin clots the levels of ampicillin were higher after intermittent administration than after constant infusion (3). The AUC_{IF} levels and the absolute levels in IF in our study were very similar to each other without any influence of mode of administration.

The levels in serum were sufficient for treating susceptible bacteria with all tested drugs. In IF, however, the obtained levels were adequate for cephradine, cefuroxime, cefotaxime, and cefoxi-

tin. Cefoperazone yielded levels in IF that were not sufficient for treating, for example, *Pseudomonas* sp. infections (Table 1).

Our conclusions from this study are that the penetration of an antibiotic into the subcutaneous IF is inversely correlated to the serum protein binding of a drug when the penetration is measured as AUC_{IF} divided by AUC_s. As no differences in AUC_{IF} were shown to be due to the mode of administration, this might indicate that such factors as pain on injection, risk of thrombophlebitis, or technical reasons might decide the mode of administration.

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