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Concentrations of Various Antibiotics in Serum and Fluids Accumulated in Diffusion Chambers Implanted in Various Sites in Rabbits

A. GEORGOPOULOS* AND E. SCHÜTZE

Sandoz Forschungsinstitut, Gesellschaft m.b.H., 1235 Vienna, Austria

Diffusion chambers with Millipore membranes were implanted in soft tissue, kidneys, and peritoneal and pleural cavities of rabbits. Single doses of azlocillin, cefazolin, and gentamicin were injected intramuscularly and ampicillin was administered orally 2-5 weeks after implantation. The concentrations of the respective drugs in simultaneously collected samples of fluid from each diffusion chamber were measured and compared with concentrations found at the same time in serum. All chambers were tolerated well, and the method proved to be effective for collecting data on the distribution of drugs throughout the body.

Several authors have described the implantation of various types of tissue cages in soft tissue of dogs, rabbits, and rats; in kidneys, prostate, and liver of dogs; and in the peritoneal cavity of rabbits to study the distribution of antimicrobial drugs in tissue fluids (11, 14).

Recently we described another type of tissue cage, a diffusion chamber with filters (Millipore Corp.) which, due to its small size, can be implanted in various sites of the rabbit. The construction of the chambers and their implantation in soft tissue and kidneys of rabbits, as well as their use in studying concentrations of different antibiotics in fluids gained from the chambers, have been described recently by our group (A. Georgopoulos et al., Infection, in press; G. Laber et al., Infection, in press).

This paper deals with the measurement of antibiotic concentrations in serum and fluids obtained simultaneously from chambers implanted in soft tissue, kidneys, peritoneal cavity, and the pleural cavity, a site heretofore not examined.

MATERIALS AND METHODS

Animals. Female rabbits (chinchilla hybrids) weighing 3.5 to 4.5 kg were used. They were kept in stainless-steel cages at a room temperature of 22°C, were fed a standard diet, and had free access to drinking water.

Diffusion chambers. The diffusion chamber consisted of a plastic ring (14-mm outer diameter, 10-mm inner diameter) closed on each side by a Millipore filter of a pore size of 0.45 μm which is provably impermeable to cells (Laber et al., Infection, in press). The chamber has a volume of 0.2 ml. For these studies it was modified to permit withdrawal of fluids at the various sampling times by the addition of two catheters, each 6 cm long, with an internal diameter of 0.6 mm, as described by Georgopoulos et al. (Infection, in press).

Implantation procedure. Each rabbit used in this study had chambers implanted in the following sequence: both kidneys, peritoneal and pleural cavities, and soft tissue. The rabbits were anesthetized with Halotane. The implantation of the diffusion chamber in the kidney has been described in detail (Georgopoulos et al., Infection, in press). Immediately after implantation of the kidney chambers, a chamber was introduced into the peritoneal cavity with the catheters being passed through the abdominal wall into the subcutis where they were anchored. The incision was sutured. To implant a chamber in the pleural cavity, the animal was then fixed laterally and an incision 1.5 cm long was made in the thorax between the eighth and ninth ribs (intercostal thoracotomy). Through this incision a chamber was placed in the pleural cavity between the lung and the diaphragm. The ends of the catheters were anchored in the subcutis. The muscles and the skin were sutured.

The subcutaneous chamber was the last to be implanted. A skin incision was made in the lumbar region, and a chamber was inserted far enough under the skin so that the catheters lay in the subcutis.

All the catheters were closed by using stainless-steel pegs. After a healing period of 2 weeks, the animals which showed no abnormal symptoms were used for the experiments.

Analysis of chamber fluids: The composition of soft tissue interstitial fluid (STIF) and renal interstitial fluid (RIF) of rabbits has been determined previously (Schmook et al., Infection, in press). Using the same methods, the analysis of the peritoneal fluid (PF) and pleural fluid (PLF) was done in five healthy rabbits 3 weeks after implantation of a chamber.

Antibiotics, dosage, and administration. Groups of four rabbits were used for each of the following antibiotics: azlocillin—Bayer, Germany, batch no. 4302 B, 50 mg/kg of body weight, intramuscular injection; cefazolin—Eli Lilly & Co., batch no. FF 7AB68C, 30 mg/kg of body weight, intramuscular

injection; gentamicin sulfate, Biochemie Kundl, Austria, batch no. 770653, 3 mg/kg of body weight, intramuscular injection; ampicillin trihydrate, pure substance, Biochemie Kundl, Austria, batch no. 40, 50 mg/kg of body weight, oral administration.

Assay procedure of drug concentration and statistical evaluation. At 0.5, 1, 2, 4, and 8 h after injection, 5 μl (the catheter volume) was withdrawn and discarded; a 20-μl sample (about 1/10 of the total volume) was then withdrawn for determination of the antibiotic concentration. Serum samples were taken at the same time points.

The drug concentrations were determined by using a micro-agar diffusion method (8). The standard curves for the antibiotics were prepared in the appropriate fluid from control chambers and serum, respectively. The test strains and the detectable limits for the different drugs were as follows: *Sarcina lutea* ATCC 9341 for ampicillin (0.2 μg/ml) and azlocillin (0.9 μg/ml), *Staphylococcus aureus* ATCC 6538 P for cefazolin (2 μg/ml), and *Staphylococcus epidermidis* ATCC 12228 for gentamicin (0.06 μg/ml).

By measuring the diffusion rate in vitro of antibiotics through the Millipore filter, no loss of activity due to binding to the membrane could be observed (Schmook et al., Infection, in press).

The values of the area under the curve for the compounds in each body fluid were computed by using the trapezoid rule for integration and adding an estimated part for the period beyond the 8-h time point by assuming a first-order elimination. The differences between the areas under the curve were evaluated by a two-way analysis of variance. The differences between the concentrations in chamber fluids were calculated by analysis of variance with repeated measurements for the time factor.

RESULTS

All the implanted diffusion chambers were tolerated well. The chamber in the pleural cavity, up to 5 weeks after implantation, was not observed to be encapsulated or fixed to the pleura. The composition of STIF, RIF, PF, and PLF from chambers implanted 3 weeks earlier is shown in Table 1.

The analyses of STIF and RIF of rabbits and the stabilization of the components have been described (Schmook et al., Infection, in press); the values for PF and PLF were also done by Schmook (personal communication).

The concentrations of the tested antibiotics in body fluids are listed in Table 2; the ratios of the areas under the concentration versus time curves are in Table 3.

Serum levels of azlocillin administered in a dose of 50 mg/kg intramuscularly reached a peak of more than 40 μg/ml 15 min after injection, decreasing to about 0.4 μg/ml at 4 h. Drug concentrations in STIF, PF, and PLF resulted in similar curves which increased up to 2 h with peak concentrations between 7.8 and 10 μg/ml. They decreased after 2 h, with detectable concentrations between 0.9 and 1.9 μg/ml at 8 h. The concentrations in the RIF peaked also at 2 h at a level of 15.6 μg/ml and decreased far more slowly, reaching an 8-h level at 7.4 μg/ml.

The examination of cefazolin gave a different picture. The serum level peaked 15 min after intramuscular (i.m.) injection of 30 mg/kg and was much higher (more than 90 μg/ml) than that of azlocillin. It also decreased to 0.3 μg/ml after 4 h. The concentrations in the different body fluids gained from the chambers were again similar. They peaked between 2 and 4 h with concentrations between 7.4 and 8.7 μg/ml and remained at levels between 4 and 6.5 μg/ml at the 8-h time point, where the concentration in RIF was the highest one.

The mean values for gentamicin were determined after injection of 3 mg/kg. The mean peak concentration in serum was 7.4 μg/ml 30 min after injection, decreasing to a concentration of about 0.3 μg/ml at 8 h. Gentamicin levels in STIF, PF, and PLF peaked 2 h after injection between 2.1 and 3.0 μg/ml, whereas the mean value in RIF reached a maximum peak of about 3.6 μg/ml at 4 h. After 8 h the RIF concentration remained the highest one, measuring 2.5 μg/ml, followed by PLF at 1.5 μg/ml, STIF at 1.4 μg/ml, and PF at 0.7 μg/ml.

After oral administration of ampicillin, serum levels were detectable for up to 8 h. The results are shown in Table 2. In contrast to profiles obtained after parenteral administration of the other β-lactam antibiotics, the serum level declined more slowly over a longer period. In serum this drug peaked at 10.6 μg/ml after 1 h and was still detectable at 8 h at an amount of 0.5 μg/ml. Peak values in RIF, PF, and PLF were reached at 2 h at 3.0, 1.7, and 2.5 μg/ml, respectively, whereas the STIF level did not peak until 4 h at 3.0 μg/ml.

The statistical evaluation revealed significant differences between the drug concentration curves in serum and chamber fluids but, due to

TABLE 1. *Composition of fluids obtained from chambers implanted in various sites in rabbits*

Fluid	Total protein (g/100 ml)	Albumin (g/100 ml)	Sodium (mVal/liter)	Potassium (mVal/liter)	Calcium (mVal/liter)
STIF	3.0 ± 0.5	2.3 ± 0.4	136.0 ± 5.0	5.2 ± 0.5	5.16 ± 0.5
RIF	4.34 ± 0.9	2.79 ± 0.5	139.4 ± 3.4	4.13 ± 1.0	4.46 ± 0.6
PF	3.87 ± 0.2	3.39 ± 0.1	144.6 ± 0.9	3.7 ± 0.1	5.57 ± 0.1
PLF	4.18 ± 0.1	3.25 ± 0.1	136.5 ± 1.2	4.2 ± 0.4	5.45 ± 0.1

TABLE 2. *Serum and tissue levels of different antibiotics in rabbits after intramuscular or oral administration, respectively (mean values in μg/ml ± SEM[a])*

Antibiotic dose/administration	Body fluid	Level after administration at:					
		0.25 h	0.5 h	1.0 h	2.0 h	4.0 h	8.0 h
Azlocillin (50 mg/kg, i.m.)	Serum	41.7 ± 3.5	34.3 ± 3.5	24.5 ± 5.2	7.3 ± 1.4	0.4 ± 0.3	0.0 ± 0.0
	STIF	ND[b]	3.8 ± 0.8	7.8 ± 0.7	10.2 ± 1.2	5.5 ± 0.6	0.9 ± 0.4
	RIF	ND	5.1 ± 1.8	7.6 ± 1.1	15.6 ± 4.8	9.6 ± 3.9	7.4 ± 3.1
	PF	ND	1.8 ± 0.7	5.2 ± 1.2	7.8 ± 0.9	4.8 ± 0.8	0.9 ± 0.5
	PLF	ND	2.1 ± 0.7	6.3 ± 1.6	9.0 ± 1.7	4.6 ± 0.5	1.9 ± 0.0
Cefazolin (30 mg/kg, i.m.)	Serum	92.9 ± 16.1	80.2 ± 10.0	49.3 ± 5.9	15.4 ± 2.1	0.3 ± 0.3	0.0 ± 0.0
	STIF	ND	0.5 ± 0.3	4.5 ± 0.8	7.4 ± 0.9	7.8 ± 0.8	4.6 ± 0.3
	RIF	ND	0.9 ± 0.4	3.8 ± 1.4	8.5 ± 1.1	8.8 ± 1.7	6.5 ± 0.8
	PF	ND	1.3 ± 0.7	4.0 ± 1.8	7.5 ± 2.1	8.4 ± 1.6	3.9 ± 0.9
	PLF	ND	1.3 ± 0.7	4.0 ± 1.1	8.7 ± 1.1	8.0 ± 1.4	4.7 ± 0.9
Gentamicin (3 mg/kg, i.m.)	Serum	6.8 ± 0.8	7.4 ± 0.3	5.7 ± 0.2	3.3 ± 0.2	1.2 ± 0.3	0.3 ± 0.2
	STIF	ND	0.7 ± 0.1	1.6 ± 0.1	3.0 ± 0.2	2.7 ± 0.1	1.4 ± 0.2
	RIF	ND	0.4 ± 0.1	1.3 ± 0.6	2.8 ± 1.3	3.6 ± 1.8	2.5 ± 1.0
	PF	ND	0.3 ± 0.1	1.1 ± 0.3	2.1 ± 0.5	2.2 ± 0.7	0.7 ± 0.5
	PLF	ND	0.6 ± 0.2	1.4 ± 0.4	2.5 ± 0.5	2.5 ± 0.7	1.5 ± 0.6
Ampicillin (50 mg/kg, orally)	Serum	3.7 ± 2.2	6.8 ± 3.6	10.6 ± 3.3	5.9 ± 2.6	1.7 ± 0.8	0.5 ± 0.3
	STIF	ND	<0.25	1.1 ± 0.6	2.0 ± 1.3	3.0 ± 0.6	1.5 ± 0.4
	RIF	ND	<0.25	0.9 ± 0.5	3.0 ± 2.3	2.6± 0.6	1.3 ± 0.1
	PF	ND	<0.25	0.9 ± 0.7	1.7 ± 0.9	1.6 ± 0.4	0.7 ± 0.1
	PLF	ND	0.3 ± 0.0	0.9 ± 0.1	2.5 ± 0.4	2.0 ± 0.3	1.0 ± 0.2

[a] SEM, Standard error of the mean.
[b] ND, Not done.

TABLE 3. *Ratios of the areas under the concentration versus time curves of four antibiotics in different body fluids*

Antibiotic	Serum	Ratio (chamber fluid/serum)			
		RIF	STIF	PLF	PF
Azlocillin	1	2	0.8	0.6	0.7
Cefazolin	1	1.3[a]	0.5	0.6	0.4
Gentamicin	1	1	1.1	0.5	0.5
Ampicillin	1[b]	1.9[b]	1.5[b]	1.1	0.6

[a] Significantly higher ($P < 0.005$) than STIF, PLF, or PF.
[b] Significantly higher ($P < 0.01$) than PF.

the large standard deviations between individual animals and the small number of animals used, no significant differences could be shown by comparing the various chamber fluids. Only among the concentrations at the various sampling times for each chamber fluid were time dependent statistically significant changes found. Statistical evaluation of the areas under the curve showed only a significance of RIF versus STIF, PLF, and PF after administration of cefazolin. After ampicillin, the areas under the curve of serum, RIF, and STIF were significantly higher than PF (Table 3).

DISCUSSION

So far there are no reports describing tissue cages or chambers for collecting fluid from the pleural cavity of any animal or RIF from rabbits to determine drug concentrations. We are not aware of any reports of pharmacokinetic studies of azlocillin using tissue cages. There are few reports on the pharmacokinetic profile of cefazolin in rabbits (2, 10, 15). Peterson et al. (15) used Visking tubing to measure serum and STIF levels after repeated intramuscular administration of 30 mg of cefazolin per kg. They described values which are in accordance with ours when taking into consideration only the levels measured before the second injection. However, Carbon and co-workers (2), using silastic tubing, presented a profile of cefazolin, 30 mg/kg injected once intramuscularly to rabbits, which is quite different from our findings. The serum and STIF concentrations measured in their experiments were far lower, about 1/3 of our values. In particular, the course of the STIF level curve is in disaccordance with the curves found by Peterson and co-workers (15) and by us. Our experiments revealed STIF concentrations which increased up to 4 h, then declined very slowly for the next 4 h. Carbon et al. (2) found a permanent increase of the STIF levels for up to 12 h which, however, never reached the amounts that we could measure. Gerding et al. (10), using perforated plastic balls and the same dosage and route of administration of cefazolin as we did, determined the concomitant concentrations in serum and peritoneal fluid. Their results agree with ours. Although concentrations in RIF of rabbits have not yet been published, those in dogs have been reported by Eickenberg (7).

Pharmacokinetic data from serum and cage fluid of gentamicin in rabbits have been reported (4, 9, 10, 12, 13). Using different types of tissue cages as well as different experimental designs, the results of Gerding and his group (10) are similar to ours although we determined somewhat higher concentrations of gentamicin in serum as well as in STIF and PF. Norrby et al. (13) used stainless-steel cages and silicon cylinders for measuring STIF concentrations. They reported peak levels between 4 to 8 h after administration whereas we could find maximum levels at 4 h. Compared with the doses reported here, Carbon et al. (4) in general measured higher concentrations of gentamicin in serum and higher and longer lasting levels in STIF with delayed peaks. Gentamicin levels in serum and STIF of dogs have been reported by Chisholm et al. (5, 6).

To our knowledge only one report has been published so far dealing with ampicillin levels in serum and STIF of rabbits. Administering about 70 mg/kg, Carbon and co-workers (3), using silastic capsules, measured a maximum concentration of about 3 μg/ml, as we did. In contrast to our results, their STIF level peaked later (at 6 h) and remained detectable for more than 12 h. Other reports of ampicillin concentrations in STIF of rats after oral administration (1) as well as after intravenous or i.m. injection in dogs (6, 7, 18) have been published.

The delayed occurrence of peak concentrations in fluids gained from the tissue cages in comparison to those obtained from our diffusion chambers is obvious in the experiments with gentamicin and also in the investigations of Carbon et al. with cefazolin (2). The delay seems to depend on the different techniques as well as on the longer periods of time the tissue cages have been implanted, in particular in the subcutis, before they are used for experiments. Ryan (16) and Ryan and Mason (17) referred to this phenomenon and suggested that reaction of the surrounding tissue to the implanted cages produced a diffusion barrier. They showed a relationship between the drug level measurable in cage fluids and the length of time the cages were implanted before being used. Prolonged implantation often results in the appearance of a relatively impermeable fibrous mass (16). We use our diffusion chambers within 2 to 5 weeks after implantation, and this may account for results different from those who use their cages many weeks or months after implantation.

Our chamber system is a novel effective method to measure drug concentrations in PLF and other body fluids. Although from these preliminary experiments only few statistically proved results are available, we believe that this technique can produce biologically relevant data from various sites at consecutive time points in the same animal.

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