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Antibiotic penetration into the infected knee

A rabbit experiment

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We investigated the diffusion of penicillin-G, cloxacillin, clindamycin, and netilmicin into synovial fluid and membrane in rabbits. Purulent arthritis was induced in the right knee of each rabbit by inoculation of *Staphylococcus aureus* phage type 3C, whereas sterile saline was injected into the left knee to serve as a control. Two days later, concentrations of antibiotics were determined in serum, synovial fluid, and membrane after an intramuscular single dose. All four drugs diffused readily into infected joints, whereas the corresponding concentrations in the normal joints were 2–3 times lower. Clindamycin showed the highest intraarticular penetration, cloxacillin the lowest. The lower penetration of cloxacillin corresponded to its higher protein binding in rabbit serum. Considering the sufficient local concentrations achieved, parenteral treatment obviates the need for local instillation of these antibiotics.

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Introduction

Because local instillation of antibiotics in septic arthritis may be potentially harmful to the joint, parenteral administration of these drugs is usually preferred (Argen et al. 1966). It is important therefore to know the extent of diffusion of antibiotics into the joint space, particularly when the joint is infected.

We have studied the penetration of penicillin-G, cloxacillin, clindamycin, and netilmicin into normal and infected joints using a rabbit arthritis model that was established in our laboratory (Riegels-Nielsen et al. 1986)

Materials and methods

Twenty-four 4-month-old albino rabbits (Ssc:CPH), weighing 2–3 kg, were used for the study. The knees were inoculated by injecting 0.5 ml of sterile saline or a *Staphylococcus aureus* suspension through the patellar ligament via a 26-gauge syringe. Each rabbit was inoculated with staphylococci in the right knee, whereas the saline-injected left knee served as a control (Riegels-Nielsen et al. 1986). Each dose of bacteria contained approximately 10^3 colony-forming units prepared as described in the previous paper (Riegels-Nielsen et al. 1986).

Forty-eight hours later, purulent arthritis had developed in the right knee, as manifested by the clinical signs of red, swollen, and warm joints with marked reduction of voluntary movement of the joint. *S. aureus* could be grown from aspirates of all the infected knees, whereas no growth could be detected in blood cultures or in aspirates from the control knees.

Forty-eight hours after inoculation, the drug studies were carried out. The antibiotics were administered in the following single dosages: Penicillin-G, 250,000 IE/kg (150 mg/kg); cloxacillin, 100 mg/kg; clindamycin, 10 mg/kg; and netilmicin, 2 mg/kg, respectively.

The penicillins were diluted in phosphate-buffered saline (PBS, pH=7.2), clindamycin and netilmicin in sterile saline. All the antibiotics were prepared fresh for each day of experiment. The antibiotics were administered i.m., 6 rabbits per drug. Two rabbits were killed by an overdose of Nembutal 1, 2, and 4 h after antibiotic administration, respectively. Blood for serum antibiotic concentration determination was obtained by ear-vein puncture at the following intervals after antibiotic administration (number of rabbits sampled): 0.5 h (6), 1 h (6), 2 h (4), 3 h (2), and 4 h (2). Before death, synovial fluid was sampled from both knees of each rabbit by intraarticular injection of 2 ml of Ringer-lactate, which was aspirated again 3 min later (Morgan et al. 1965). Immediately after death, both knees of each rabbit were opened and synovial tissue obtained.

Antibiotic concentrations were measured by disc-diffusion bioassay. The assay had the following coefficients of variation: netilmicin, 0.8%; penicillin, 2.7%; cloxacillin, 4%; clindamycin, 10%. The following bacterial strains were incorporated: *K. pneumoniae* for netilmicin, and *S.lutea* for the three other antibiotics.

Synovial tissue samples were homogenized and diluted in PBS and antibiotic concentrations determined in the solution. Standard curves were generated in pooled rabbit serum, Ringer's lactate solution or PBS, for serum, synovial fluid and synovial tissue, respectively.

The area under the concentration curve was estimated by the trapezoidal rule for the interval 0–4 h after antibiotic administration.

Results

The synovial fluid drug concentrations in the infected joints were generally higher than those of the control joints (Tables 1–4). For all the drugs, although in the lower range for netilmicin, the serum concentrations corresponded to concentrations achievable in human beings after clinically relevant dosages. In both knees the synovial fluid concentrations exceeded the minimal inhibitory concentrations (MIC's) for most common pathogens isolated from purulent arthritis. At the time of the study, i.e., 48 h after inoculation, exudation in the infected joints was minimal (Riegels-Nielsen et al. 1986). Therefore, the technique of injecting Ringer lactate into the knee to mimic synovial fluid resulted in the same fluid volume in the normal as in the infected knees. Apart from cloxacillin, the drugs were eliminated from the infected knees at roughly the same rate as in serum. The elimination of cloxacillin, in contrast, appeared to be somewhat slower than in serum. A similar delay in local drug elimination for cloxacillin seemed to occur in the synovial tissue.

Synovial membrane concentrations in noninfected knees were only measurable after 1 h for penicillin in 1 rabbit, and in no animals for the three other drugs.

The 0–4 h areas under the concentration curves (AUC) from synovial fluid and serum (Table 5) illustrated the twofold-to-threefold superior penetration of all four drugs in infected joints as compared with noninfected joints. The order of penetration for the drugs was clindamycin > netilmicin > penicillin-G > cloxacillin.

Table 1. Concentrations of penicillin in serum, synovial fluid, and synovial tissue of control (C) and infected (I) knees after a single dose of 150 mg/kg in rabbits. Mean (range)

Time (h)	Antibiotic concentration (mg/l)				
	Serum	Fluid		Tissue	
		C	I	C	I
0.5	32.6 (23.3–45.0)	ND	ND	ND	ND
1	22.9 (11.6–34.6)	7.6 (2.6–12.6)	20.3 (3.9–36.7)	0.5 (0.5)	1.0 (0.8–1.1)
2	9.5 (3.8–15.0)	2.3 (0.9–3.6)	2.0 (1.8–2.2)	–	–
3	2.2 (0.8–3.7)	ND	ND	ND	ND
4	1.0 (0.0–2.0)	0.2 (0.0–0.3)	0.4 (0.2–0.5)		

ND, not determined.

Table 2. Concentrations of cloxacillin in serum, synovial fluid, and synovial tissue of control (C) and infected (I) knees after a single dose of 150 mg/kg in rabbits. Mean (range)

Time (h)	Antibiotic concentration (mg/l)				
	Serum	Fluid		Tissue	
		C	I	C	I
0.5	55.3 (35.2–71.4)	ND	ND	ND	ND
1	36.4 (26.0–53.1)	1.6 (1.6)	3.1 (1.8–4.3)	–	–
2	15.3 (9.8–19.5)	1.4 (0.0–2.8)	3.8 (2.0–5.5)	–	8.5 (8.4–8.6)
3	5.4 (4.1–6.7)	ND	ND	ND	ND
4	1.3 (0.0–2.0)	–	1.8 (1.8)	–	6.7 (5.1–8.2)

ND, not determined.

Table 3. Concentrations of clindamycin in serum, synovial fluid, and synovial tissue of control (C) and infected (I) knees after a single dose of 10 mg/kg in rabbits. Mean (range)

Time (h)	Antibiotic concentration (mg/l)				
	Serum	Fluid		Tissue	
		C	I	C	I
0.5	3.3 (2.8–4.3)	ND	ND	ND	ND
1	2.7 (1.9–3.7)	0.9 (0–1.8)	1.6 (1.5–4.3)	–	3.3 (3.3)
2	1.4 (1.2–1.7)	0.8 (0–1.5)	1.9 (1.3–2.4)	–	1.5 (0–3.0)
3	1.1 (1.0–1.1)	ND	ND	ND	ND
4	0.8 (0.8)	–	0.9 (0.7–1.0)	–	–

ND, not determined.

Table 4. Concentrations of netilmicin in serum, synovial fluid, and synovial tissue of control (C) and infected (I) knees after a single dose of 2 mg/kg in rabbits. Mean (range)

Time (h)	Antibiotic concentration (mg/l)				
	Serum	Fluid		Tissue	
		C	I	C	I
0.5	4.8 (3.5–6.2)	ND	ND	ND	ND
1	3.2 (2.5–4.5)	1.5 (1.1–1.8)	1.8 (1.8)	–	1.5 (0.6–2.4)
2	1.1 (0.5–1.3)	–	1.0 (0.8–1.2)	–	0.9 (0.7–1.0)
3	0.5 (0.5)	ND	ND	ND	ND
4	–	–	0.2 (0.2)	–	0.5 (0.4–0.6)

ND, not determined.

Table 5. Data on the area under the drug concentration curves in serum (s) and in synovial fluid (f) from infected (i) and from control (c) knees

	s	fc	fi	fc/s	fi/s	fi/fc
Penicillin	2741	675	1422	0.25	0.52	2.1
Cloxacillin	4578	222	636	0.05	0.14	2.9
Clindamycin	395	126	321	0.32	0.81	2.6
Netilmicin	384	90	228	0.23	0.55	2.3

Discussion

The pathogens isolated most frequently from cases of septic arthritis are *S. aureus* or *S. epidermidis*, beta-hemolytic streptococci, pneumococci, *H. influenzae*, and *Neisseria* sp. (Argen et al. 1966, Harlow et al. 1975, Newman 1976). Gram-negative bacteria of the *Enterobacteriaceae* group or *Pseudomonas aeruginosa* are found with increasing frequencies in patients with immune defects (Newman 1976). The antibiotics tested in our study are active against most of these pathogens in vitro. Their activity in vivo depends upon their penetration of the focus of infection, i.e., the joint space and surrounding tissue. Because infection causes pathophysiologic changes in the tissue involved, it is important to evaluate the pharmacokinetics of the antibiotics under such conditions. Our infection model seems reliable and reproducible (Riegels-Nielsen et al. 1986).

The consequences of infection for diffusion of antibiotics into the rabbit knee were clearly discernible in our study. All four antibiotics achieved concentrations in the synovial fluid of the infected joints that were 2–3 times the corresponding concentrations in the normal joints. At the dosages chosen, which were comparable to usual clinical doses, all four antibiotics appeared in sufficiently high concentration in the synovial fluid and membrane of the infected knees to show activity severalfold above the MIC's for most pathogens isolated from septic arthritis. Further, the antibiotics remained in the infected joints for a considerably longer period than in the control, normal joints.

The antibiotic concentrations measured in the synovial membrane may not reflect the amount of microbiologically active drug present. First, a certain degree of tissue binding may take place. Secondly, the actual concentrations of the drugs are obscured by homogenization of the tissue: for antibiotics distributed in extracellular fluid only (penicillins and aminoglycosides), which constitutes 20 per cent of the homogenate, the concentrations assayed were fivefold lower than in extracellular fluid. For lipophilic drugs (clindamycin), which are distributed both intracellularly and extracellularly, the concentrations assayed are the same as present in the homogenate. The latter condition probably correlates better with the clinical situation because *S. aureus* is located both extracellularly and intracellularly.

Little information is available on the intraarticular penetration of the antibiotics studied. The cloxacillin concentrations in synovial fluid were lower than those reported by Parker et al. (1971), but their patient received cloxacillin for a considerably longer time and at higher dosages. Data on intraarticular penetration of gentamicin and tobramycin correspond to those of netilmicin in our study (Chow et al. 1971, Dee et al. 1973, March et al. 1974). Morgan et al. (1965), in a similar rabbit model as ours, found penicillin concentrations in the infected joints to be lower than those in the noninfected knees. However, they performed their drug studies 7 days after inoculation of the rabbits in contrast to our 2 days. This may imply that penetration into infected joints decreases with the duration of inflammation (Nelson 1971). The increased diffusion in the early stages of infection is clearly of benefit to the patient. The subsequent decrease in diffusion of antibiotics indicates that dosages ought to be increased in later stages of purulent arthritis.

Clindamycin showed the highest penetrabililty, probably explained by the lipophilicity of this drug. Another factor of importance appears to be the degree of protein binding. For cloxacillin, which is 78–96 per cent bound to rabbit serum protein (Craig et al. 1980), only 14 per cent of the amount of drug in serum could be detected in the synovial fluid of the infected knee (Table 5). However, cloxacillin elimination was delayed both in synovial fluid and in synovial membrane, indicating binding of the drug locally. Protein binding did not appear to influence the diffusion of the three other drugs tested, which all are less than 60 per cent bound in rabbit serum (Craig et al. 1980).

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