NOTES

Effect of Probenecid on Penetration of Oxacillin into Fibrin Clots In Vitro

RACHEL D. LEE, JOHN L. BRUSCH,¹* MICHAEL J. BARZA, AND LOUIS WEINSTEIN

Infectious Disease Service, Department of Medicine, New England Medical Center Hospital, Boston, Massachusetts 02111

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Probenecid significantly enhanced the in vitro penetration of oxacillin into fibrin clots suspended in rabbit serum or normal saline.

The delivery of antibiotic in adequate concentration to the site of the offending organism is a major requisite in the chemotherapy of infection. The results of a previous investigation suggested that probenecid might alter the dynamics of movement of oxacillin into fibrin loci in vivo, initially retarding and then enhancing its penetration (1). The present study explores some aspects of this interaction in vitro.

Materials and methods. Oxacillin (Bristol Laboratories, Syracuse, N.Y.) was dissolved in rabbit serum (Microbiological Associates, Bethesda, Md.) or in normal saline solutions in a concentration of 40 $\mu\text{g/ml}$. These solutions were distributed in flasks in volumes of 100 ml. To one of a pair of identical antibiotic solutions, 0.6 ml of probenecid (Merck, Sharpe and Dohme, West Point, Pa.) were added to produce a concentration of 144 $\mu\text{g/ml}$. The pH of the serum solutions was adjusted to 7.4 by maintaining them in an atmosphere of 5% CO_2 throughout the study. The pH of some of the normal saline solutions, originally pH 6.9, was adjusted to 6.0 or 8.0 by the use of HCl or NaOH.

Fibrin clots were prepared by a previously described method (3). Thirty-six clots were placed in each flask; they were incubated at 37 C with frequent agitation. At 1, 2, 4, and 6 h, nine clots and 1 ml of the solution were removed from each flask and assayed for antibiotic content by a method described elsewhere (2). Standards for antibiotic assay were prepared in rabbit serum, normal saline, and fibrin clots dissolved in trypsin (Difco Laboratories, Detroit, Mich.), both with and without probenecid. The content of each sample was interpreted from the corresponding standard curve.

¹ Present address: U.S. Public Health Service Hospital, Brighton, Mass. 02135.

Fibrin clots were dissolved by streptokinase-streptodornase (Lederle Laboratories, Pearl River, N.Y.), and their albumin content was determined by an AutoAnalyzer.

The effect of probenecid on the protein binding of oxacillin was studied by an equilibrium dialysis technique described elsewhere (2). Oxacillin in a concentration of 40 $\mu\text{g/ml}$ was initially present on the serum side in approximately half the dialysis chambers and on the Krebs-Ringer side in the others. Additional chambers were prepared identically except that probenecid was added to either the serum or Krebs-Ringer solution at a concentration of 150 $\mu\text{g/ml}$. A total of 26 chambers were studied, 13 with and 13 without probenecid. Standard solutions were prepared in serum and in Krebs-Ringer solution, both with and without probenecid. The degree of protein binding was calculated in the usual manner (2).

All statistical comparisons were performed by an unpaired *t* test.

TABLE 1. *Percentage of penetration of oxacillin into fibrin clots suspended in serum (pH 7.4)^a*

Condition	Penetration (%)			
	1 h ^b	2 h	4 h	6 h
With probenecid	19.6 $\pm$ 1.0	29.4 $\pm$ 1.3	37.8 $\pm$ 2.8	44.5 $\pm$ 3.8
Without probenecid	13.4 $\pm$ 0.9	21.8 $\pm$ 1.3	31.0 $\pm$ 1.5	39.8 $\pm$ 5.0
P value ^c	<0.001	<0.001	<0.001	>0.05

^a Each value represents mean and standard error of results for 27 clots (three experiments).

^b Time of sampling.

^c Difference in percentage of penetration with and without probenecid.

TABLE 2. *Percentage of penetration of oxacillin into fibrin clots suspended in saline solution at pH 6.0, 6.9, and 8.0^a*

pH	Probenecid ^b	Penetration (%)			
		1 h ^c	2 h	4 h	6 h
6.0	+	47.4 ± 2.6	75.3 ± 1.8	94.9 ± 2.8	90.8 ± 4.2
	0	46.0 ± 1.9	69.6 ± 3.8	100.6 ± 3.2	86.2 ± 3.8
6.9 ^d	+	50.1 ± 1.8	63.7 ± 1.9	84.2 ± 2.7	83.2 ± 1.9
	0	34.0 ± 0.1	37.9 ± 2.0	58.7 ± 0.2	56.5 ± 1.2
8.0 ^d	+	95.3 ± 1.8	111.2 ± 2.8	116.9 ± 1.1	140.4 ± 4.9
	0	84.2 ± 2.0	98.3 ± 3.1	109.4 ± 1.8	105.8 ± 2.2

^a Values represent mean and standard error of results for nine fibrin clots (one experiment).

^b +, With probenecid; 0, without probenecid.

^c Time of sampling.

^d Differences in percentage of penetration with and without probenecid are significant ($P < 0.01$) at all time periods with solutions at pH 6.9 and 8.0.

Results. The percentage of penetration was defined as the concentration of antibiotic in fibrin clots expressed as a percentage of that simultaneously present in the solution. The percentage of penetration of oxacillin from serum into fibrin clots was significantly enhanced at 1, 2 and 4 h by the presence of probenecid (Table 1). Similarly, the percentage of penetration was higher in the presence of probenecid at 1, 2, 4, and 6 h in solutions of saline at pH 6.9 and 8.0 but not in those at pH 6.0 (Table 2).

After 6 h of incubation, the concentration of oxacillin in solutions of serum containing probenecid was $32.0 \pm 1.3 \mu\text{g/ml}$ (mean and standard error of three values); for serum without probenecid the level was $36.1 \pm 2.8 \mu\text{g/ml}$. The albumin content of fibrin clots immersed in rabbit serum was 0.4 g/100 ml after 1 h, and 0.85 g/100 ml after 6 h, with no difference noted between solutions containing and lacking probenecid.

Probenecid was found to reduce the protein binding of oxacillin in serum from $75.7 \pm 1.0\%$ to $65.2 \pm 1.5\%$ ($P < 0.01$). The concentration of antibiotic in the serum at the end of dialysis ranged from 24 to 32 $\mu\text{g/ml}$.

Discussion. The results of this study indicate that, in concentrations encountered therapeutically in man (4), probenecid enhances the penetration of oxacillin into fibrin clots from both protein-containing and protein-free solutions. The reasons for this phenomenon are not clear. In general, the distribution of penicillins between serum and fibrin loci is influenced by the degree of protein binding of the antibiotic (2). The fact that probenecid enhanced penetra-

tion in saline solutions, however, indicates that the observations cannot be adequately explained in terms of alterations of protein binding of the antibiotic. The discrepancy between the results in saline solutions at pH 6.9 or 8.0 (significant enhancement) and those at pH 6.0 (no effect) suggests that the extent of ionization of the drugs may play a role; the pKa of oxacillin, however, is 2.8 (5) and that of probenecid is 3.4 (4), so that both agents should be almost fully ionized at pH 6.0.

We have no satisfactory explanation for these findings. It is of interest, however, to note that they are similar to those observed during h 4 and 5 of the in vivo study (1).

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