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Effect of Probenecid on Cerebrospinal Fluid Concentrations of Penicillin and Cephalosporin Derivatives

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Probenecid may elevate the cerebrospinal fluid (CSF) concentration of penicillin G by inhibiting the excretion of organic acids from CSF. We have studied this phenomenon with various penicillin and cephalosporin derivatives. Penicillin concentrations were determined in rabbits under steady-state conditions before and after intravenous probenecid administration. With both low-dose and high-dose probenecid, CSF penicillin levels increased two to three times as did CSF concentration as a percentage of serum level. The same probenecid effect was consistently demonstrated in animals with experimental pneumococcal meningitis. Probenecid likewise increased the CSF concentration of ampicillin, carbenicillin, nafcillin, cephacatrile, and cefazolin. Probenecid may prove useful in certain bacterial infections where high CSF antibiotic levels are necessary.

The low cerebrospinal fluid (CSF) concentrations achieved by penicillin and cephalosporin derivatives may present a problem in the treatment of infections of the nervous system. Probenecid may be effective in raising the CSF concentration of penicillin G (3, 4, 5). This effect is mediated by inhibition of renal excretion, competitive serum protein binding, and competitive inhibition of carrier-mediated organic acid excretion by choroid plexus (and possibly other peri-CSF systems) (1, 4, 5).

This study investigates the effect of probenecid on CSF concentrations of various penicillin and cephalosporin derivatives and examines the phenomenon in experimental pneumococcal meningitis.

MATERIALS AND METHODS

Preparation of animals. A total of 13 New Zealand white rabbits weighing 1 to 2 kg were prepared in the following manner. At least 24 h prior to each experiment, six aluminum wood screws (0.5 by 2 inches or 1.27 by 5.08 cm) were introduced to a depth of approximately 1 mm into the outer table of cranial vertex and 2 mm lateral to sagittal suture in positions over the frontal, parietal, and occipital cortex. Care was taken not to introduce the screw tip intracranially. Then, by using Dura Base dental acrylic, a half turnbuckle (2.5 by 1.8 cm) was cemented to the implanted wood screws.

On the day of each experiment, animals were anesthetized with pentobarbital (25 mg/kg intravenously [i.v.]) and ether, and PE90 polyethylene catheters were placed in the femoral artery and vein. Then, by inserting a machine bolt (0.25 by 4 inches or 0.635 by 10.16 cm) into the threaded female receptacle of the turnbuckle, the animal's head was rigidly but painlessly secured to a modified stereotaxic frame. Next, the posterior alanto-occipital membrane was exposed surgically, and the cisterna magna was carefully punctured using a Quincke spinal needle (3.5 by 25 gauge) mounted in a geared electrode introducer. Clear CSF was withdrawn for cell count. Bloodless CSF was consistently obtained by this method. Animals were maintained under light pentobarbital sedation resulting in no significant changes in arterial blood gases.

Conduct of experiments. A constant i.v. infusion of the various antibiotics was administered, at the dosages listed in Table 1, by using a Sage infusion pump (model 352) via the femoral venous catheter. Serial serum and CSF samples were taken from the indwelling spinal needle and arterial catheter at 30-min intervals over the 8-h period of infusion. Probenecid was administered by i.v. bolus every 60 min (doses of either 10 or 20 mg per kg per h) starting 4 h after the beginning of antibiotic infusion; steady-state levels of antibiotic were obtained in serum and CSF within 1 to 2 h. Thus each animal was used as its own control negating a predictable intra-animal variation.

A total of 480 antibiotic levels were determined by agar well-diffusion techniques. A *Bacillus subtilis* spore solution (0.125 ml) was added to 100 ml of antibiotic medium no. 1 (Difco) and poured into plates. Wells (0.75 by 0.75 cm) were cut into agar and filled with approximately 0.04 ml of specimens. Zone of inhibition was measured and compared to a standard curve. The standard curve was determined by dissolving known concentrations of antibiotic in pooled rabbit serum and saline (zone sizes for identical concentrations of antibiotic dissolved in rabbit CSF and saline were found to be equal; thus all CSF concentrations were calculated from standard curves with saline diluent).

Production of meningitis. Washed *Streptococcus*

pneumoniae type 3 (10^8) from a fresh 18-h culture were injected into the CSF via the cisterna magna of five rabbits. The needle was removed. Twelve to 18 h later, animals were lethargic and febrile to 40.5 C. Examination of the CSF revealed purulent meningitis with 4,000 to 6,000 leukocytes, with 95% polymorphonuclear leukocytes and glucose of less than or equal to 40 mg/100 ml. Quantitative cultures before therapy were 10^7 organisms per ml. Penicillin was administered as described above with probenecid injections beginning 4 h after the start of constant penicillin infusion.

Statistical analysis. Data for each animal were processed in the same manner. At least eight samples were assayed before and during probenecid therapy. All values obtained after the steady-state was reached are expressed as a mean ± standard deviation for each experiment. Differences between groups were determined by Student's *t* test.

RESULTS

Probenecid was found to increase the CSF concentrations of all penicillin and cephalosporin derivatives tested (Table 1).

Effect of probenecid on CSF penicillin concentrations in normal animals. Penicillin did not accumulate in the CSF over the 8-h experimental period. An animal given a constant i.v. penicillin infusion without probenecid was found to have a relatively constant CSF penicillin concentration (mean, 0.3 to 0.4 μg/ml) over the 8-h course (Table 2). CSF level expressed as a percentage of serum level was also at 0.4% for 8 h. When low-dose probenecid (10 mg per kg per h) was administered during hours 5 to 8 of penicillin infusion (0.5 million U per kg per h), no change in serum levels occurred but CSF penicillin concentration increased: 0.9 ± 0.2 to 1.7 ± 0.3 μg/ml ($P < 0.01$). The CSF concentration expressed as a percentage of serum levels likewise increased, from 0.2 ± 0.1% to 0.6 ± 0.1% ($P < 0.01$) (Fig. 1). When a higher dose of probenecid (20 mg per kg per h) was administered during hours 5 to 8, an increase in serum penicillin levels from 62 ± 15 μg/ml to 89 ± 15 μg/ml ($P < 0.01$) occurred, whereas CSF concentrations increased from 0.3 ± 0.1 μg/ml to 0.9 ± 0.3 μg/ml ($P < 0.01$). This increase was greater than could be explained by the rise in serum concentration, i.e., an increase in CSF level as percentage of serum level from 0.5 ± 0.1% to 1.1 ± 0.2% ($P < 0.01$). The CSF concentration of penicillin, expressed as percentage of serum concentration, as well as the response to probenecid were both remarkably consistent over a wide range of serum levels (Fig. 1 and Table 2).

Effect of probenecid on CSF penicillin concentrations in experimental meningitis. An animal with experimental *S. pneumoniae* meningitis (CSF leukocytes 6,400, 95% polymorphonuclear leukocytes with CSF glucose less than 40 mg/100 ml) received a constant i.v. infusion of penicillin for 6 h (Table 1). Serum and CSF concentrations expressed as percentage of serum concentration remained essentially unchanged over the 6-h infusion, and there was no evidence of accumulation of drug in CSF. The action of probenecid was exaggerated in animals with meningitis. When probenecid was added during hours 5 to 8 of penicillin infusion, serum penicillin levels increased from 20 ± 2 μg/ml to 36 ± 11 μg/ml ($P < 0.01$) and CSF penicillin levels increased from 0.3 ± 0.1 μg/ml to 1.5 ± 0.7 μg/ml ($P < 0.01$). Again, this increase in CSF level was greater than could be explained by increase in serum level alone since CSF level expressed as percentage of serum concentration increased from 1.5 ± 0.2% to 4.5 ± 0.1% (Fig. 2). This phenomenon was consistent with two other similar experiments: CSF increased from 2.1 ± 0.7 μg/ml to 14.2 ± 6.4 μg/ml and from 1.2 ± 0.3 μg/ml to 6.7 ± 1.0 μg/ml ($P < 0.01$). The CSF concentration expressed as a percentage of serum level also increased, in both experiments, from 3.9 ± 1.7% to 6.6 ± 1.8% ($P < 0.02$) and from 1.7 ± 0.7% to 4.3 ± 0.3% ($P < 0.01$).

Effect of probenecid on penicillin and cephalosporin derivatives. Serum levels of ampicillin rose from 63 ± 17 μg/ml to 115 ± 21 μg/ml ($P < 0.01$) and CSF levels rose from 0.9 ± 0.4 μg/ml to 2.5 ± 0.4 μg/ml ($P < 0.01$) with probenecid administration. As with penicillin, the CSF level expressed as percentage of serum level also increased (1.4 ± 0.3% to 1.9 ± 0.1%, $P < 0.01$) suggesting a direct effect on CSF excretion. Carbenicillin responded to probenecid administration in a similar fashion: serum levels increased from 44 ± 5 μg/ml to 60 ± 8 μg/ml ($P < 0.01$); CSF levels increased from 0.9 ± 0.8 μg/ml to 2.0 ± 0.4 μg/ml ($P < 0.05$); and

TABLE 1. *Antibiotics administered via constant i.v. infusion*

Antibiotic	Dosage (per kg per h)
Pencillin G	0.25 and 0.50 mU
Ampicillin	100 mg
Carbenicillin	100 mg
Nafcillin	100 mg
Cefazolin	100 mg
Cephacatrile	100 mg

TABLE 2. *Effect of probenecid on CSF plus serum concentration of penicillin and cephalosporin derivatives*[a]

Animals	Regimen of animal treatment	Mean serum antibiotic concn (μg/ml ± SD)			Mean CSF antibiotic concn (μg/ml ± SD)			Mean CSF level expressed as % of serum level (% ± SD)		
		1 to 4 h	5 to 8 h	*P* value	1 to 4 h	5 to 8 h	*P* value	1 to 4 h	5 to 8 h	*P* value
1	Pencillin control (no probenecid)	54 ± 9	92 ± 31	<0.01	0.3 ± 0.07	0.4 ± .07	NS	0.4 ± 0.1	0.4 ± 0.1	NS
2	Penicillin + probenecid (10 mg per kg per h)	345 ± 145	92 ± 40	NS	0.9 ± 0.2	1.7 ± 0.3	<0.01	0.5 ± 0.1	1.1 ± 0.2	<0.01
3	Penicillin + probenecid (20 mg per kg per h)	62 ± 15	89 ± 15	<0.01	0.3 ± 0.1	0.9 ± 0.3	<0.01	0.5 ± 0.1	1.1 ± 0.2	<0.01
4	Penicillin control with meningitis	66 ± 11	50 ± 13	NS	2.4 ± 0.5	2.5 ± 0.5	NS	4.4 ± 0.1	5.1 ± 0.1	NS
5	Penicillin + probenecid with meningitis	20 ± 2	36 ± 11	<0.01	0.3 ± 0.1	1.5 ± 0.7	<0.01	1.5 ± 0.2	4.5 ± 0.1	<0.01
6	Penicillin + probenecid with meningitis	55 ± 9	235 ± 27	<0.01	2.1 ± 0.7	14.2 ± 6.4	<0.01	3.9 ± 1.7	6.6 ± 1.8	<0.02
7	Penicillin + probenecid with meningitis	72 ± 19	152 ± 10	<0.01	1.2 ± 0.3	6.7 ± 1.0	<0.01	1.7 ± 0.7	4.3 ± 0.3	<0.01
8	Ampicillin + probenecid	63 ± 17	115 ± 21	<0.01	0.9 ± 0.4	2.5 ± 0.4	<0.01	1.4 ± 0.3	1.9 ± 0.1	<0.01
9	Carbencillin + probenecid	44 ± 5	60 ± 8	<0.01	0.9 ± 0.8	2.0 ± 0.6	<0.05	1.0 ± 0.3	2.9 ± 0.1	<0.01
10	Nafcillin + probenecid	64 ± 10	108 ± 19	<0.01	0.6 ± 0.4	1.0 ± 0.3	<0.05	0.8 ± 0.6	0.9 ± 0.3	NS
11	Cefazolin + probenecid	143 ± 23	238 ± 37	<0.01	1.0 ± 0.2	1.8 ± 0.5	<0.01	0.7 ± 0.2	0.8 ± 0.2	NS
12	Cephacatrile + probenecid	107 ± 10	223 ± 2	<0.01	1.2 ± 0.3	2.8 ± 0.8	<0.01	1.2 ± 0.2	1.3 ± 0.2	NS

[a] Infusion of antibiotic, 6 h. SD, Standard deviation; NS, not significantly different. Antibiotic alone, 1 to 4 h; antibiotic plus probenecid, 5 to 8 h.

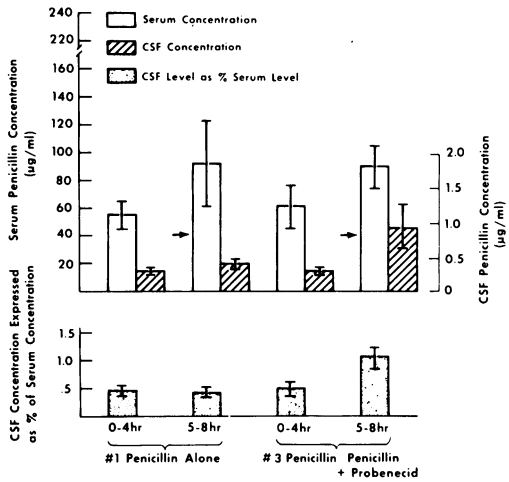

FIG. 1. *Effect of probenecid on CSF and serum concentrations of penicillin in normal rabbits.*

CSF levels expressed as percentage of serum levels increased from 1.0 ± 0.3% to 2.9 ± 0.1% ($P < 0.01$). A constant nafcillin infusion resulted in an increase in serum from 64 ± 10 µg/ml to 108 ± 19 µg/ml ($P < 0.01$) and in CSF levels from 0.6 ± 0.4 µg/ml to 1.0 ± 0.3 µg/ml ($P < 0.05$) during probenecid administration. Cefazolin responded to probenecid with a similar increase in serum from 143 ± 23 µg/ml to 238 ± 38 µg/ml and in CSF levels from 1.0 ± 0.2 µg/ml to 1.8 ± 0.5 µg/ml. Cephacatrile responded to probenecid with an increase in serum concentration from 106 ± 10 µg/ml to 222 ± 2 µg/ml ($P < 0.01$). It is of interest that although animals receiving nafcillin, cefazolin, and cephacatrile had increases in CSF concentrations, the CSF level as a percentage of serum level did not increase significantly (Table 2).

DISCUSSION

These data demonstrate that probenecid was effective in elevating the CSF concentration of penicillin in normal rabbits and rabbits in whom experimental *S. pneumoniae* meningitis had been produced. This increase was greater than could be explained by the action of probenecid on serum penicillin levels alone, since the CSF level expressed as a percentage of serum level increased in both normal rabbits and rabbits with meningitis. Expression of CSF concentration as a percentage of serum concentration was a concept also used by Fishman to emphasize the effect of probenecid directly on CSF concentrations (5).

Since variation in serum and CSF levels between animals could be expected, experiments were designed so that each animal served as his own control; i.e., the steady-state relationship between CSF and serum levels that developed in hours 1 to 4 (before probenecid administration had begun) was compared with that which developed in each individual animal during hours 5 to 8 (during probenecid administration). In spite of this inter-animal variation, the phenomenon of increasing CSF concentration in excess of increase in serum concentration was consistently present with penicillin. Others have shown in dogs that CSF penicillin levels increase over the course of a 4-h infusion and suggest that the drug accumulates in the CSF compartment (6). This did not occur with this rabbit model. Animals 1 and 4 demonstrated that CSF levels and CSF levels expressed as percentage of serum levels reached a steady state after 1 to 2 h even in the face of meningitis.

Animals 2 and 3 offered us some insight into the dosage of probenecid necessary to effect CSF penicillin levels. A dose of 10 mg per kg per h was effective in increasing CSF concentrations but did not effect serum concentrations. A probenecid dosage of 20 mg per kg per h resulted in an increase in serum and CSF levels and CSF level as a percentage of serum level. This suggests that the threshold probenecid dose necessary to produce a measurable effect on CSF organic acid excretion may be lower than that necessary for an effect on the renal tubules. The serum levels used in this experiment, however, were high and a small effect on tubular secretion could have been obscured.

The effect of probenecid on CSF ampicillin and carbenicillin concentrations was similar to

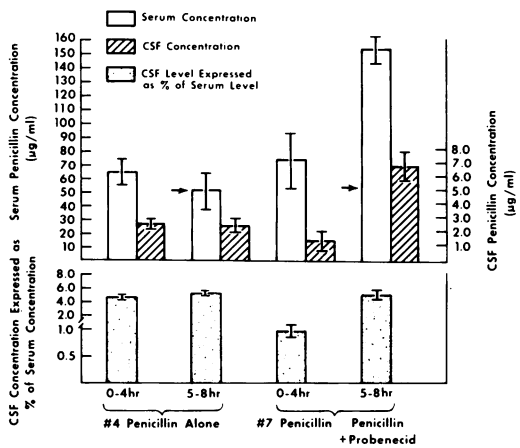

FIG. 2. *Effect of probenecid on CSF and serum concentrations of penicillin in experimental meningitis.*

that of penicillin as reflected by an increase in the CSF level expressed as a percentage of serum level. This increase in CSF level by probenecid expressed as a percentage of serum level was not observed for nafcillin, cefazolin, and cephacatrile, although CSF levels did rise in proportion to serum. One could not predict the effect of probenecid on CSF organic acid excretion (as manifest by an increase in CSF concentration expressed as a percentage of serum level) by noting the effect on the renal tubule as reflected by an increase in serum level.

The mechanism underlying the effect of probenecid on CSF was investigated primarily by Fishman (5). In his experiments with three dogs, he determined that probenecid increased the CSF penicillin level and CSF-to-plasma ratio in three ways: it (i) raised blood level, (ii) inhibited active transport of penicillin from CSF, and (iii) competitively bound serum protein with resultant increase in freely diffusable penicillin. Although he demonstrated increased CSF concentrations, brain levels expressed as a percentage of serum concentrations actually decreased with administration of probenecid to rats (5). Dixon et al. (4) reported ventriculocisternal perfusion studies showing selective, active transport of penicillin from ventricular system to blood and its inhibition by *para*-aminohippurate and diodrast (both organic acids similar to probenecid). The organic acid secretion capacity of the CSF system is localized in choroid plexus epithelium in the lateral, third and fourth ventricles and probably in extrachoroidal sites (1, 2, 4, 5). Dewhurst (3) administered probenecid to 16 patients receiving penicillin and one patient receiving ampicillin for treatment of neurosyphilis and found that probenecid induced a sixfold increase in CSF penicillin concentrations and a threefold increase in CSF ampicillin concentrations. He did not report serum levels.

It is of interest that the action of probenecid on CSF penicillin levels was not diminished but in fact slightly greater in the presence of meningeal inflammation, even with much higher initial CSF penicillin concentrations. This suggests that the organic acid secretion system of CSF is relatively more important in determining CSF penicillin concentration in the presence of meningeal inflammation since more penicillin crosses the blood-CSF barrier and is available for carrier-mediated excretion. An alternative explanation could be that removal of drug by bulk flow might be impeded by the inflammatory response in the subarachnoid space, thereby leaving only the carrier-mediated active transport system for clearance of drug from CSF. Fishman (5) showed that diffusion was an unimportant mechanism for penicillin egress for CSF. Inhibition of this system with probenecid could, therefore, have a more profound effect on increasing CSF concentrations.

The therapeutic implication of this phenomenon may be minimal for penicillin alone since merely by administering larger doses, higher CSF concentrations can be achieved. With other drugs, the achievable CSF levels may only be marginal with standard doses, i.e., *Haemophilus influenzae* treated with ampicillin, *Pseudomonas/Proteus* with carbenicillin or ticarcillin. Here the addition of probenecid may have some potential therapeutic usefulness.

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