

THE ENHANCEMENT OF β -LACTAM ANTIBIOTIC THERAPY BY NOVOBIOCIN

A. KATHRINE MILLER, EVMARIE CELOZZI and BARBARA ANN PELAK

Merck Institute for Therapeutic Research, Rahway, New Jersey 07065, USA

(Received for publication April 25, 1977)

Novobiocin demonstrates an effect similar to that of probenecid (the "probenecid effect") in enhancing the therapeutic efficacy of antibiotics excreted mainly by the renal tubules. The ability of cefoxitin, cephalixin, cephalothin and penicillin G to protect mice against infection with *Salmonella schottmuelleri* was enhanced 2- to 3-fold when the animals were given oral doses of either probenecid or of novobiocin. The efficacy of cephaloridine, excreted mainly by glomerular filtration, was not enhanced by either probenecid or by novobiocin.

Novobiocin has been reported by FUJIMOTO *et al.*¹⁾ to resemble probenecid in that it blocks anionic transport in the chicken at the peritubular side of the renal tubule cell. These workers stated that the spectrum of materials blocked and the site of action of novobiocin were similar to, but not identical with, that of probenecid.

In man probenecid inhibits the renal tubular excretion and enhances the serum concentrations of penicillin G^{2,3,4)}, cefoxitin⁵⁾, cephalixin^{6,7)} and cephalothin^{8,9)}. Cephaloridine is excreted mainly by glomerular filtration and therefore the "probenecid effect" for this antibiotic is not marked¹⁰⁾.

We have reported increased antibiotic concentrations in the serum of mice given probenecid with certain antibiotics¹¹⁾. Relatively large amounts of probenecid are required (in relation to the dose for man), but enhancement of antibiotic concentrations was demonstrated both for cephalothin and cefoxitin, but not for cephaloridine. Mouse protection tests were used to show that the increased concentrations in serum are therapeutically useful. Treatment with probenecid enhanced the therapeutic activity of penicillin, cephalothin and cefoxitin, but not of cephaloridine.

The experiments described here were performed in order to determine whether the reported probenecid-like activity of novobiocin could be demonstrated as an enhancement of therapeutic effects.

Materials and Methods

Antibiotics

Commercially available cephalixin, cephaloridine, cephalothin (all Eli Lilly & Co.) and penicillin G (Upjohn Co.), and laboratory lots of cefoxitin sodium and of novobiocin were put into solution with sterile water. Probenecid (Merck & Co.) was dissolved in 2.5 N NaOH and then diluted with water for use (final pH, 9.0).

Animals

Female Charles River CDI mice (Charles River Breeding Laboratories, Wilmington, Mass.) were used. Average weight at the time of testing was 19~21 g.

Mouse Protection Test

Salmonella schottmuelleri was used as the infecting agent in the majority of the experiments. The culture, designated as Merck Sharp & Dohme stock culture number 3010, is maintained in the lyophilized state. For each test a vial was restored with Brain Heart Infusion broth (BHI; BBL) and, after

24 hours growth at 37°C was transferred, again into BHI broth, for incubation overnight at 37°C. This 16-hour culture was diluted 1/50, again in BHI broth. This suspension, containing about 5×10^6 cells/ml, was used to infect the mice intraperitoneally. The challenge dose of 0.5 ml contained 5~50 LD₅₀ doses (LD₅₀=the number of cells lethal to 50% of infected, untreated animals).

A standard plate count procedure was used to determine the numbers of organisms that were in the challenge dose, and a virulence titration was included in each test to calculate the LD₅₀. At the time of infection and again 6 hours later, drug treatments were given by the routes indicated in Tables 1~3. Ten mice were used for each of the two-fold antibiotic dilutions tested. Survival data obtained on the seventh day after infection were used to calculate the ED₅₀ (amount of antibiotic necessary to protect 50% of the infected animals), and the LD₅₀. For these tests, about 2,500 mice were used.

Statistical Procedures

The LD₅₀ and ED₅₀ values were calculated by the method of KNUDSEN and CURTIS.¹²⁾ Logs of the approximate 95% confidence limits were obtained by calculating $(\bar{m} \pm Qw)$ where \bar{m} is the average of the logs of the ED₅₀ values, Q is a factor depending on the number of assays combined (for two assays Q=1.25; for three assays Q=0.68; for four assays Q=0.49 and for five assays Q=0.38) and w is the difference between the highest and the lowest values of the logs of the individual ED₅₀ values included in \bar{m} . The antilogs of the figures so obtained are the lower and higher 95% confidence limits. The mean effective dose and its approximate limits, therefore, are expressed as ED₅₀, antilogs $(\bar{m} \pm Qw)$. This formula was suggested by Dr. J. L. CIMINERA of Merck & Co., Inc.

Results

Table 1 compares the effects of probenecid and of novobiocin on the ability of cefoxitin to protect mice against infection with *Salmonella schottmuelleri*. It can be seen that when 500 mg/kg probenecid

Table 1. Effect of probenecid and of novobiocin on cefoxitin therapy in mice

Compound and dosage route	ED ₅₀ ^{a)} in mg/kg × 2 in Test No.			
	1	2	3	Mean ^{b)}
Cefoxitin, sc	36.9	25	26.5	29.4
Cefoxitin, sc+probenecid, po ^{c)}	10.9		10.9	10.9
Cefoxitin, sc+novobiocin, po ^{c)}	10.9	11.7		11.3

a) Mice infected intraperitoneally with 7~19 LD₅₀ doses of *Salmonella schottmuelleri* 3010 in broth at 0 hour. Therapy at 0 and 6 hours. Ten mice at each of the 2-fold cefoxitin concentrations tested.

b) Geometric mean.

c) Probenecid 500 mg/kg/dose.
Novobiocin 250 mg/kg/dose

Table 2. Titration of the probenecid effect on therapy of *Salmonella schottmuelleri* in mice

Antibiotic	ED ₅₀ mg/kg/sc dose with Probenecid mg/kg/po dose				Probenecid Ratio 0 : 500
	0	31.2	125	500	
Cefoxitin, sc	26.6	12.5	10.0	10.9	2.4
Cephalothin, sc ^{a)}	78.2	40.7	33.2	25.6	3.0
	ED ₅₀ mg/kg/sc dose with Novobiocin mg/kg/po dose				Novobiocin ratio 0 : 250
	0	62.5	125	250	
Cefoxitin, sc	25.0	17.7	13.3	11.8	2.1

a) Mean of 2 tests.

Table 3. Effect of probenecid and novobiocin on the ability of antibiotics to protect mice against *Salmonella schottmuelleri*

Antibiotic ^{a)}	ED ₅₀ ^{b)} mg/kg/dose with			Ratio	
	Saline (S)	Probenecid (P)	Novobiocin (N)	S/P	S/N
Cefoxitin	29.0 (22~39) ^{c)}	10.9 (10.9)	11.3 (10~12)	2.7	2.6
Cephalexin	40.7 (10~160)	21.9 (16~30)	23.7 (16~35)	1.9	1.8
Cephalothin	92 (64~134)	32.1 (29~35)	55.7 (31~108)	2.9	1.7
Penicillin G	41.2 (33~51)	12.8 (11~15)	16.1 (12~22)	3.2	2.6
Cephaloridine	7.4 (6~9)	6.2 (5~7)	6.8 (5~10)	1.2	1.1

^{a)} Given subcutaneously, except cephalexin orally, 0 and 6 hours after intraperitoneal infection with 7~50 LD₅₀ doses.

^{b)} Geometric mean of 2~4 tests for each value.

^{c)} Approximate 95% confidence limits.

was given orally at the time of each subcutaneous treatment with cefoxitin, the antibiotic ED₅₀ value was reduced 2.5 times. A similar reduction in ED₅₀ was shown when 250 mg/kg novobiocin was given orally. Neither probenecid nor novobiocin alone protected any mice when given at these concentrations to infected, otherwise untreated mice.

The use of 500 mg/kg probenecid had been established by earlier work with penicillin G. This dose is about one-third the reported oral toxic (LD₅₀) dose for mice, *i.e.*, 1,666 mg/kg¹³⁾. The 250 mg/kg novobiocin dose is about one-quarter the 962 mg/kg LD₅₀ oral dose for mice¹⁴⁾. To determine whether this amount of novobiocin was required to show the "probenecid effect" seen in Table 1, titration experiments were run. Various amounts of either probenecid or of novobiocin were given orally to mice infected with *Salmonella schottmuelleri* and treated subcutaneously with cefoxitin. The effect of probenecid on cephalothin therapy also was titrated. The data are shown in Table 2. It can be seen that the efficacy of these antibiotics in this infection increases up to two- to three-fold with the increasing concentrations of probenecid or novobiocin. Maximum protection by the antibiotics was seen when the highest doses of probenecid or novobiocin were given, therefore, these doses were used in all further tests.

To determine whether novobiocin also enhances the efficacy of other antibiotics, cephalexin, penicillin G and cephaloridine were used as the therapeutic agents. Table 3 shows that the reduction of ED₅₀ values obtained with probenecid also is seen for novobiocin, though not always to the same extent. As anticipated the enhancing effect was seen with cefoxitin, cephalexin, cephalothin and penicillin G, but not with cephaloridine. The effect on cephalexin seems borderline; the variability of the endpoints for saline treatment extends the 95% confidence limits of this control group to points overlapping those of the two test groups. The end points and confidence limits for probenecid and novobiocin are, however, quite similar.

Discussion

Data presented here indicate that novobiocin does indeed demonstrate the probenecid effect of enhancing the therapeutic efficacy of antibiotics excreted by the renal tubules. Because novobiocin itself is an antibiotic agent, the possibility was considered that the observed enhancement represented a synergistic response. VERWAY *et al.*¹⁵⁾ and MILLER *et al.*¹⁶⁾ studied the interaction of novobiocin and penicillin both *in vitro* and *in vivo* against *Micrococcus pyogenes* (*Staphylococcus aureus*). They con-

cluded that, although synergism and antagonism were occasionally demonstrated, novobiocin and penicillin usually interacted in an additive manner. They suggested that a combination of novobiocin and penicillin could be used to broaden the spectrum of activity of the individual agents. To demonstrate this they infected mice with a mixture of a penicillin-resistant *Staphylococcus* and *Streptococcus*. An amount of penicillin G that was ineffective against the *Staphylococcus*, but could protect against the *Streptococcus*, was combined with an amount of novobiocin ineffective against the *Streptococcus*, but effective against the *Staphylococcus*. All the mice given the mixed infection were protected by this combination of individually ineffective concentrations of novobiocin and penicillin G. In the light of the data presented here, the earlier report of MILLER *et al.*¹⁶⁾ might now be said to demonstrate not only a broadened spectrum of antibiotic activity but, in addition, the possible enhancement of penicillin action by novobiocin.

References

- 1) FUJIMOTO, J. M.; J. J. LECH & R. ZAMIATOWSKI: A site of action of novobiocin in inhibiting renal tubular transport of drugs in the chicken. *Biochem. Pharmacol.* 22: 971~979, 1973
- 2) BOGER, W. P.; J. O. BEATTY, F. W. PITTS & H. F. FLIPPIN: The influence of a new benzoic acid derivative on the metabolism of para-aminosalicylic acid (PAS) and penicillin. *Ann. Int. Med.* 33: 18~31, 1950
- 3) BURNELL, J. M. & W. M. M. KIRBY: Effectiveness of a new compound, benemid, in elevating serum penicillin concentrations. *J. Clin. Invest.* 30: 697~700, 1951
- 4) WALDO, J. F. & J. T. TYSON: The effect of benemid on plasma penicillin levels. *J. Lab. Clin. Med.* 37: 272~277, 1951
- 5) GOODWIN, C. S.; E. B. RAFERTY, A. D. GOLDBERG, H. SKEGGS, A. E. TILL & C. M. MARTIN: Effects of rates of infusion and probenecid on serum levels, renal excretion and tolerance of intravenous doses of cefoxitin in humans: Comparison with cephalothin. *Antimicrob. Agents & Chemoth.* 6: 338~346, 1974
- 6) BRAUN, P.; J. R. TILLOTSON, C. WILCOX & M. FINLAND: Cephalexin and cephaloglycin activity *in vitro* and absorption and urinary excretion of single oral doses in normal young adults. *Appl. Microb.* 16: 1684~1694, 1968
- 7) MEYERS, B. R.; K. KAPLAN & L. WEINSTEIN: Cephalexin: Microbiological effects and pharmacologic parameters in man. *Clin. Pharmacol. & Therapeut.* 10: 810~816, 1969
- 8) LEE, C-C.; E. B. HERR, Jr. & R. C. ANDERSON: Pharmacological and toxicological studies on cephalothin. *Clin. Med.* 70: 1123~1138, 1963
- 9) KLEIN, J. O.; T. C. EICKHOFF, J. C. TILLES & M. FINLAND: Cephalothin: Activity *in vitro*, absorption and excretion in normal subjects and clinical observations in 40 patients. *Amer. J. Med. Sci.* 248: 640~656, 1964
- 10) KISLAK, J. W.; B. W. STEINHAEUER & M. FINLAND: Cephaloridine activity *in vitro* and absorption and urinary excretion in normal young men. *Am. J. Med. Sci.* 251: 433~447, 1966
- 11) MILLER, A. K.; E. CELOZZI, Y. KONG, B. A. PELAK, D. HENDLIN & E. O. STAPLEY: Cefoxitin, a semi-synthetic cephamycin antibiotic: *In vivo* evaluation. *Antimicrob. Agents & Chemoth.* 5: 33~37, 1974
- 12) KNUDSEN, L. F. & J. M. CURTIS: The use of angular transformation in biological assays. *J. Amer. Stat. Ass.* 42: 282~296, 1947
- 13) MCKINNEY, S. E.; H. M. PECK, J. M. BOCHY, B. B. BYHAM, G. S. SCHUCHARDT & K. H. BEYER: Benemid, (*p*-di-*n*-propylsulfamyl)-benzoic acid: Toxicologic properties. *J. Pharmacol. & Exp. Therapeu.* 102: 208~214, 1951
- 14) LARSON, E. J.; N. D. CONNOR, O. F. SWOAP, R. A. RUNNELLS, M. C. PRESTRUD, T. E. EBLE, W. A. FREYBURGER, W. VELDKAMP & R. M. TAYLOR: Novobiocin, a new antibiotic. VI. Toxicology. *Antibiot. & Chemoth.* 6: 226~230, 1956
- 15) VERWEY, W. F.; A. K. MILLER & B. J. BARON: Novobiocin-penicillin combinations. I. The antibacterial interaction between novobiocin and penicillin. *Antibiot. Ann.* 1957/1958: 31~37, 1958
- 16) MILLER, A. K.; B. J. BARON & W. F. VERWEY: Novobiocin-penicillin combinations. II. Broadened spectrum of activity of combinations of novobiocin and penicillin. *Antibiot. Ann.* 1957/1958: 38~42, 1958