STUDIES ON PROTEIN BINDING OF ANTIBIOTICS

III. EFFECT OF NOVOBIOCIN ON PROTEIN BINDING AND PHARMACOKINETICS OF CEFOPERAZONE AND CEFAZOLIN

YASUO WATANABE, TOSHIO HAYASHI, RIEKO KITAYAMA, TAKASHI YASUDA, ISAMU SAIKAWA and KIHACHIRO SHIMIZU*

Research Laboratory, Toyama Chemical Co., Ltd., Toyama, Japan
*Department of Internal Medicine, Tokyo Women's Medical College, Tokyo, Japan

(Received for publication March 23, 1981)

The drug-protein interactions between cefoperazone (CPZ) and novobiocin (NB), and between cefazolin (CEZ) and NB were investigated.

Though there was a remarkable reduction of CPZ or CEZ binding to rabbit serum and human serum albumin with increases in drug concentrations above 3.0×10^{-4} M (CPZ: 200 μg/ml, CEZ: 140 μg/ml), NB binding was not affected. In addition, when CPZ or CEZ was added to the NB solution, NB binding was not altered and remained above 90%. Therefore, it was evident that NB had a high capacity for binding to protein, compared with CPZ or CEZ. From the competitive study, the main binding site of NB to protein appeared to differ from that of CPZ or CEZ.

The CPZ or CEZ serum levels in rabbits for the simultaneous administration of NB were significantly higher than those for the control experiments, however, the NB serum levels were not greatly affected by CPZ or CEZ.

We have reported the drug-protein interactions *in vitro* and *in vivo* between antibiotics, *i.e*, between cefoperazone (CPZ) and cefazolin (CEZ)[1], and between apalcillin (APPC) and CPZ or CEZ[2]. Our reports have shown that the binding behavior of CPZ to serum protein is similar to that of CEZ, however, APPC is somewhat different from CPZ or CEZ.

In the present study, we selected novobiocin (NB)[3], which has a high serum protein binding, and investigated the drug-protein interactions between CPZ and NB, and between CEZ and NB.

Materials and Methods

Drugs

Cefoperazone (CPZ) was prepared by the Research Laboratory, Toyama Chemical Co., Ltd., Toyama, Japan, cefazolin (CEZ) by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, and novobiocin (NB) by Japan Upjohn Company Co., Ltd., Tokyo, Japan.

Animals

Japanese white adult male rabbits were used, weighing 2.0~3.0 kg.

Drug Administration

Solutions of the drugs were prepared in saline. NB in a dose of 20 mg/kg was administered alone and with 20 mg/kg of CPZ or CEZ into auricular vein.

Binding Experiments

The extent of binding of drugs to serum protein was determined by the method of centrifugal ultrafiltration. The interference by NB in the binding of CPZ or CEZ to human serum albumin was evaluated by the formula of KLOTZ *et al*[4]. The exact procedure was described in the previous report[1].

Measurement of Antibiotic Concentration

Measurement of the antibiotic concentration was performed with a high pressure liquid chromatograph (HPLC, Shimadzu LC-2). Samples were run on a column (250 mm×4 mm ϕ) of LiChrosorb RP-18 at an ambient temperature and a flow rate of 1.0 ml/minute. The mobile phase consisted of CH_3CN - 1 M CH_3COOH - 1 M $(C_2H_5)_3N \cdot CH_3COOH$ - H_2O (400: 14: 27: 559). The eluate was monitored at 254 nm. The HPLC's criteria for CPZ and CEZ were described in the previous report[1].

Results and Discussion

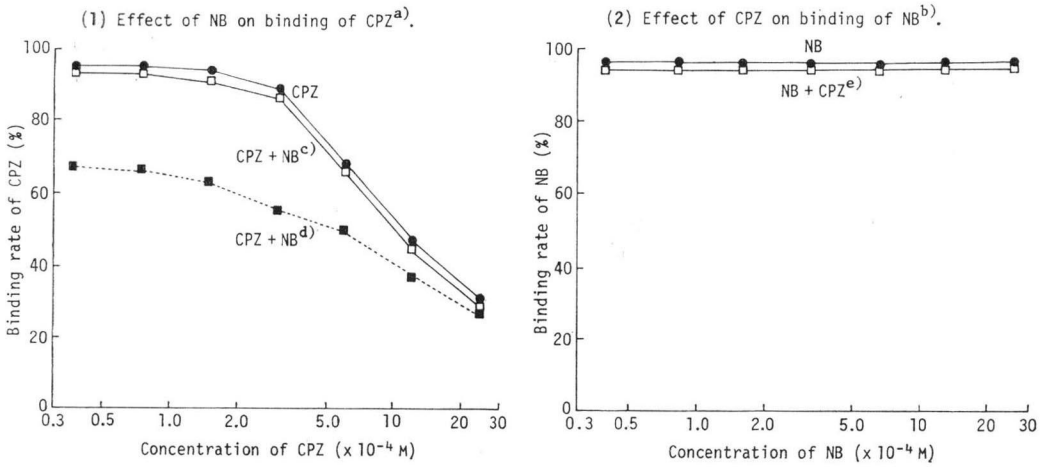
1. *In Vitro* Displacement between CPZ and NB, and between CEZ and NB

As shown in Fig. 1, the extent of binding of CPZ were determined over a range of the final concentrations of drug extending from 0.4×10^{-4} M (25 μg/ml) to 24.0×10^{-4} M (1600 μg/ml). The CPZ binding were largely independent of the concentration of drug below 3.0×10^{-4} M (200 μg/ml), but above this concentration, the binding decreased with the increases in drug concentration. Similar results were obtained for CEZ (Fig. 2). The decreased binding rates which occurred above this concentration were apparently due to the saturation of available protein binding sites.

CPZ or CEZ binding was not affected by the addition of NB (400 μg/ml). This appeared to be in agreement with results already reported by ROLINSON[5]: the addition of NB at a concentration of 500 μg/ml to serum containing cloxacillin (50 μg/ml) has no significant effect on the binding rate of cloxacillin. However, the addition of NB (1600 μg/ml) greatly reduced the binding of CPZ or CEZ. These results suggest that in the presence of 6.3×10^{-4} M NB (400 μg/ml), CPZ or CEZ is independently bound to each main binding site and in the presence of 1600 μg/ml, NB competes with the main binding site of CPZ or CEZ.

NB binding was also determined, ranging from 0.4×10^{-4} M to 25.2×10^{-4} M (25~1600 μg/ml). NB binding did not change, even at the concentration (1600 μg/ml) at which CPZ binding decreased by

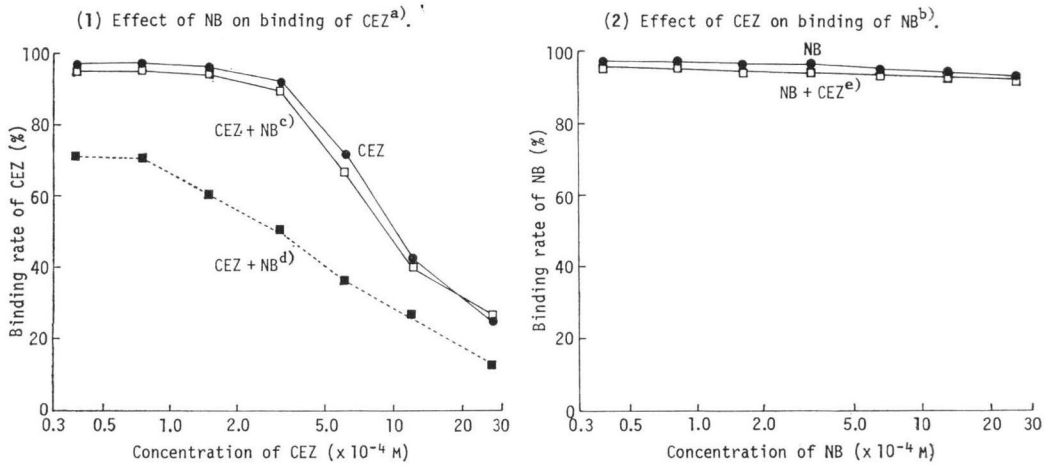
Fig. 1. Competitive binding between CPZ and NB.



Protein: Rabbit serum. Method: Centrifugal ultrafiltration.

a) Binding rate of CPZ in the absence and presence of NB.
b) Binding rate of NB in the absence and presence of CPZ.
c) NB: 6.3×10^{-4} M (400 μg/ml).
d) NB: 25.2×10^{-4} M (1600 μg/ml).
e) CPZ: 6.0×10^{-4} M (400 μg/ml).

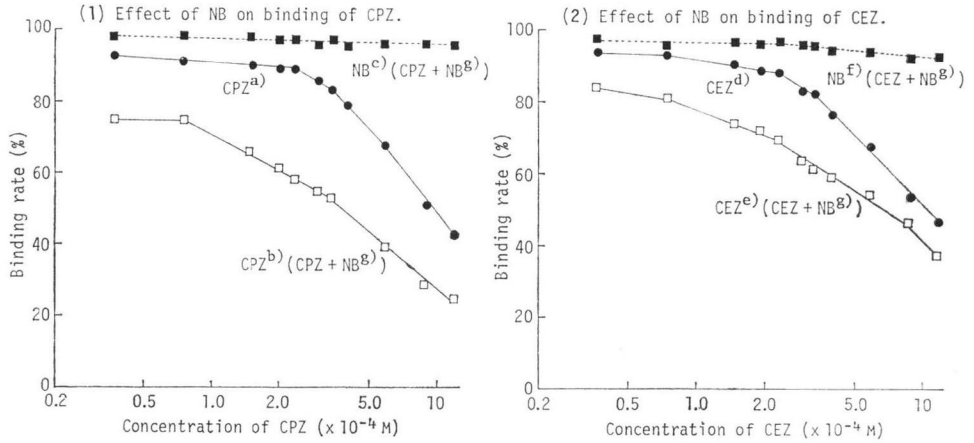
Fig. 2. Competitive binding between CEZ and NB.



Protein: Rabbit serum. Method: Centrifugal ultrafiltration.

a) Binding rate of CEZ in the absence and presence of NB.
b) Binding rate of NB in the absence and presence of CEZ.
c) NB: 6.3×10^{-4} M (400 μg/ml).
d) NB: 25.2×10^{-4} M (1600 μg/ml).
e) CEZ: 5.9×10^{-4} M (280 μg/ml).

Fig. 3. Competitive binding between CPZ and NB, and between CEZ and NB.



Protein: Human serum albumin (5.8×10^{-4} M: 4%). Method: Centrifugal ultrafiltration.

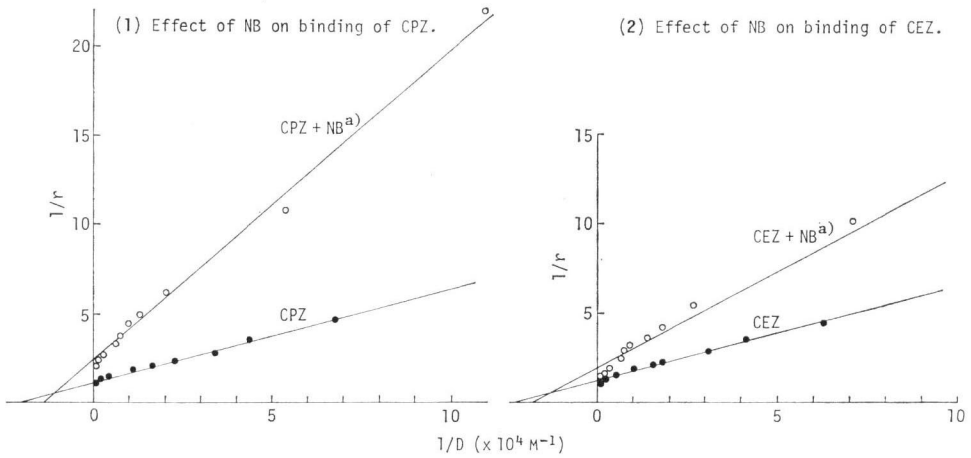
a) Binding rate of CPZ in the absence of NB.
b) Binding rate of CPZ in the presence of NB.
c) Binding rate of NB in the presence of CPZ.
d) Binding rate of CEZ in the absence of NB.
e) Binding rate of CEZ in the presence of NB.
f) Binding rate of NB in the presence of CEZ.
g) NB: 5.8×10^{-4} M (368 μg/ml).

about 60%. In addition, though CPZ (400 μg/ml) or CEZ (280 μg/ml) was added to the NB solution, NB binding was not altered and remained above 90%.

It thus became clear that NB had a high capacity for binding to protein and that its main binding site did not appear to coincide with that of CPZ or CEZ.

Further detailed investigations were performed using human serum albumin (Figs. 3, 4). CPZ

Fig. 4. KLOTZ plots for binding of CPZ and CEZ to human serum albumin.



Human serum albumin: 5.8×10^{-4} M.
r: Amount of drug per one molecule.
D: Free drug concentration.
a) NB: 5.8×10^{-4} M (368 μg/ml).

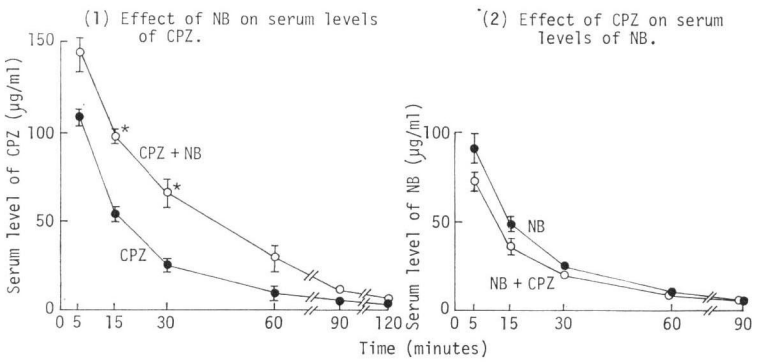
binding subsided by approximately 20~30% in the presence of 5.8×10^{-4} M NB (368 μg/ml), compared with control experiments. This trend was also observed for CEZ. These results did not coincide with those obtained for rabbit serum. In contrast, NB binding was not reduced and remained above 90% in the presence of CPZ or CEZ in all concentrations up to 11.6×10^{-4} M. The application of the formula of KLOTZ *et al.*[4] showed that both drugs partially shared the binding sites. The interference of NB in CEZ binding was somewhat less than that in CPZ binding.

In the light of our previous and present results, it is suggested that the capacity of drugs to bind to protein may be ranked, in descending order, NB, APPC, and CPZ or CEZ.

2. Alteration of Serum Levels of CPZ or CEZ by NB

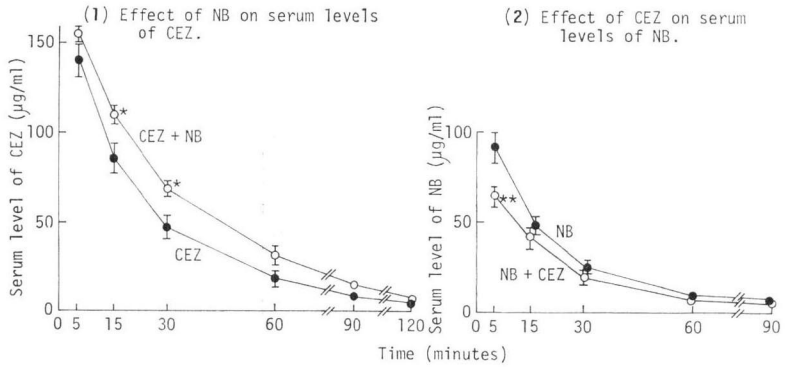
The serum levels of CPZ or CEZ in rabbits, with and without NB, were determined. The time

Fig. 5. Serum levels of CPZ and NB in rabbits.



Rabbits were intravenously dosed with CPZ (20 mg/kg) and NB (20 mg/kg), both separately and simultaneously. Each group consisted of 5 rabbits.
*: Significant difference at $P<0.01$.

Fig. 6. Serum levels of CEZ and NB in rabbits.



Rabbits were intravenously dosed with CEZ (20 mg/kg) and NB (20 mg/kg), both separately and simultaneously. Each group consisted of 5 rabbits.

* Significant difference at $P<0.05$.

** Significant difference at $P<0.01$.

course of CPZ, CEZ and NB in serum is shown in Figs. 5, 6. The levels of CPZ for the simultaneous administration of NB were significantly higher than those of the control experiments. This may be that NB inhibits the excretion of CPZ, since both drugs are mainly excreted in bile in rabbits. Similar results were obtained for CEZ, but it cannot be explained by the competition in excretion in the liver, because CEZ is mainly excreted in urine. The functioning of other mechanisms would be indicated. By way of contrast, the NB levels were not affected by the simultaneous administration of CPZ. It can be explained by the fact that NB binding was not affected by CPZ *in vitro*. However, the fact that the CEZ level at 5 minutes was significantly lower than that for the control experiment cannot be explained from the *in vitro* results.

Thus, the pharmacologic activity of drugs is altered by the simultaneous administration of other drugs. Further studies concerning drug-protein interactions, therefore, seem necessary.

References

1) Watanabe, Y.; T. Hayashi, R. Takada, T. Yasuda, I. Saikawa & K. Shimizu: Studies on protein binding of antibiotics. I. Effect of cefazolin on protein binding and pharmacokinetics of cefoperazone. J. Antibiotics 33: 625~635, 1980

2) Watanabe, Y.; T. Hayashi, R. Kitayama, T. Yasuda, I. Saikawa & K. Shimizu: Studies on protein binding of antibiotics. II. Effect of apalcillin on protein binding and pharmacokinetics of cefoperazone and cefazolin. J. Antibiotics 34: 753~757, 1981

3) Lubash, G.; J. van der Meulen, C. Bernsten & R. Tompsett: Noboviocin: a laboratory investigation. Antibiot. Med. 2: 233~240, 1956

4) Klotz, I. M.; H. Triwush & F. M. Walker: The binding of organic ions by proteins. Competition phenomena and denaturation effect. J. Am. Chem. Soc. 70: 2935~2941, 1948

5) Rolinson, G. N. & R. Sutherland: The binding of antibiotics to serum proteins. Brit. J. Pharmacol. 25: 638~650, 1965