Hiroshima Journal of Medical Sciences
Vol. 30, No. 4, December, 1981
HIJM 30-39

EFFECT OF BOVINE SERUM ALBUMIN ON THE ABSORPTION OF SULFONAMIDES IN THE RAT*)

By

Yutaka HIGASHI and Noboru YATA

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine

(Received September 2, 1981)

ABSTRACT

Effect of bovine serum albumin (BSA) on the absorption of sulfonamides was studied employing *in situ* recirculating perfusion method in the rat. The absorption rate constant of sulfamethoxazole was greatly decreased by addition of BSA to the perfusion solution. But, the decreasing effect of BSA on the absorption rate was diminished after removing BSA from perfusion solution. The decreasing effect of BSA on the absorption rate constant of sulfanilamide was not observed. The binding of sulfonamides to BSA was studied using an equilibrium dialysis method. Sulfamethoxazole was bound to large extent, whereas sulfanilamide was little bound. The bound fraction of sulfamethoxazole increased as the concentration of BSA increased. Influence of BSA on permeability of intestinal mucosa was not observed. Thus, the decreasing effcet of BSA on the absorption rate constant of sulfamethoxazole was attributed mainly to the binding to BSA which results in the decrease of unbound drug permeable to mucosal membrane. However, experimental results were slightly deviated from the theoretical value calculated by assuming that bound drug was unabsorbed from the intestinal mucosa.

INTRODUCTION

An orally administered drug must be absorbed from gastrointestinal tract to an extent and at a rate that will result in circulating drug levels sufficient to elicit a pharmacological response of desired magnitude and duration. The efficiency with which a drug is absorbed is a function of many variables[1]. Furthermore, absorption of drugs have been reported to be affected by another drugs, pharmaceutical additives or food administered concomitantly[2-11]. The interaction of drug with another drug, with nonmedicinal component of dosage form or with substances normally found in biological systems (*e.g.*, in the gastrointestinal tract) can cause the formation of complexes[8-11], resulting in the increase or decrease of drug absorption. Such complexes may be essentially unabsorbable if they are practically lipoid-insoluble and large in size. In the present study, effect of complexation to macromolecule on the absorption of drugs were investigated. As models of drug and macromolecule, sulfonamides and bovine serum albumin (BSA) were used. The absorption of drug from aqueous solution with or without BSA was studied employing *in situ* recirculating perfusion method in the rat.

*) 東　豊，矢田　登：ラットにおけるサルファ剤の吸収におよぼす牛血清アルブミンの影響

EXPERIMENTAL

Materials——Commercially available sulfanilamide and sulfamethoxazole were used without further purification. Bovine serum albumin (Fraction V) (BSA) was purchased from the Armour Pharmaceutical Company and cellophane tubing (Visking Company, 27/32 inflated diameter) was used as a dialyzing membrane. All other chemicals were analytical grade.

Procedure of In situ Absorption Experiment——Male Sprague-Dawley rats weighing 200 to 300 g were fasted for about 20 hrs prior to the experiments but water was allowed *ad libitum*. The animals were anesthetized with pentobarbital and the small intestine was exposed by a midline abdominal incision. The stomach and cecum were ligated by cannulation below the duodenum and at the upper portion of ileocecum with glass cannulae connected to polyethylene tube. The intestine was replaced in the abdomen, and incision closed. The small intestine was first cleared of particulate matter with 100 ml of drug-free saline solution previously warmed to 37°C. The perfusion solution was perfused with a cum pump (Tokyo Rika Kikai, C-16) at a rate of 5 ml/min, and was maintained at 37°C. Phosphate-citrate isotonic buffer, pH 6.5 was used as a perfusion solution.

To determine the absorption rate constant of sulfonamides, the following perfusion procedure was used. Sixty ml of perfusion solution containing drug and phenol red as a volume indicator was perfused. The initial 20 ml of perfusion solution after perfusion was discarded and remaining 40 ml was recirculated. Five min after the start of recirculation, 0.5 ml of perfusion solution was withdrawn from reservor as a 0 time sample, and then, at designated time, 0.5 ml of sample was withdrawn for 30 min. Sulfonamides and phenol red were analysed separately. Unabsorbed fraction in perfusion solution at time t, D_t, was calculated with equation 1.

$$D_t = \frac{C_t}{C_0} \times \frac{I_0}{I_t} \qquad \text{(Eq. 1)}$$

where, C_0 and C_t are drug concentrations in the perfusion solution at time 0 and t, respectively, I_0 and I_t are concentrations of phenol red used as a volume indicator, at time 0 and t, respectively. Logarithmic plot of unabsorbed fraction against time resulted in a straight line. This finding shows that the absorption obey the pseudo first-order kinetics. Then, the following equation will be derived.

$$\log D_t = \log D_0 - \frac{k_{app}}{2.303} \cdot t \qquad \text{(Eq. 2)}$$

Apparent pseudo first-order absorption rate constant, k_{app}, was calculated from the slope of the line which was obtained using least squares analysis.

Binding to Bovine Serum Albumin——BSA was dissolved in a phosphate-citrate isotonic buffer (pH 6.5) used in absorption experiments. The binding of drugs to BSA was determined with an equilibrium dialysis method employing the cellophane bag. Ten ml of the BSA solution was pipetted in a cellophane bag. The bag was placed in a glass tube containing 10 ml of the buffer solution of drug at various concentrations. After continuous horizontal shaking of 90 cycle/min for 8 hrs at 37°C in a water bath shaker, the concentration of drug outside the bag was measured. It took 6 hrs to establish a dialysis equilibrium. In the preliminary experiments, it was determined that no sulfonamides was bound to the cellophane tubing over the range of concentration studied.

Results were plotted according to the method of Scatchard[12], using the relationship:

$$\frac{r}{C_f} = K \cdot n - K \cdot r \qquad \text{(Eq. 3)}$$

where, r is number of moles of drug bound per mole of protein, C_f is molar concentration of unbound drug, K is association constant (litter/mole), and n is number of drug-binding sites per molecule of protein. The value of 69000 was used as the molecular weight of BSA[13]. Bound fraction, β, of sulfonamides in the perfusion solution (total (bound and unbound) concentration of 1 mM) is unable to be determined directly by the method of equilibrium dialysis, then was calculated using the n and K at a respective concentration of BSA, employing equation 4.

$$\beta = \frac{C_b}{C_t} = \frac{n \cdot P_t + C_t + K^{-1} - \{(n \cdot P_t + C_t + K^{-1})^2 - 4n \cdot P_t \cdot C_t\}^{1/2}}{2 \cdot C_t} \qquad \text{(Eq. 4)}$$

where, C_b and C_t are bound and total concentration of drug, respectively, and P_t is molar concentration of albumin.

Analytical Methods——Sulfonamides were colorimetrically assayed employing a modified Bratton-Marshall method with Tsuda's reagent[14]. Phenol red was analysed colorimetrically at 545 nm after alkalization with NaOH.

RESULTS

Effect of Bovine Serum Albumin on the Absorption of Sulfonamides through the Rat Small Intestine

Absorption of sulfonamides through rat small intestine was studied employing the *in situ* recirculating perfusion method at pH 6.5. The fraction of unabsorbed sulfamethoxazole and sulfanilamide in the recirculating perfusion solution were plotted in logarithmic scale against time. Straight lines were obtained (not shown). Furthermore, concentration-dependent change of absorption rate constant of sulfamethoxazole was not observed at a concentration range of 0.5 to 2.0 mM (Table I). These findings indicate that absorption processes of sulfonamide are performed through a passive diffusion, at the concentrations studied.

Table I. The Absorption Rate Constant of Sulfamethoxazole in Rat Small Intestine

Concn.[a] (mM)	k_{app}[b] ($min^{-1} \times 10^3$)	n[c]
0.5	12.21±0.98	5
1.0	12.05±0.45	10
2.0	13.09±0.78	5

a) initial concentration of sulfamethoxazole
b) absorption rate constant
c) number of experiments

Effect of BSA on the absorption of sulfonamides were studied at a drug concentration of 1 mM. Fig. 1 is a plot of absorption rate constant of sulfamethoxazole as a function of BSA concentration. A decrease in the absorption rate constant was observed as the BSA concentration increased. To study the physiological change of intestinal mucosa, further experiments were performed. Perfusion solution containing sulfamethoxazole (1 mM) and BSA (1.25 w/v%) was perfused for 20 min and the absorption rate constant was determined. And then, the perfusion solution was replaced with that containing drug without BSA, followed by the same perfusion procedure to determine the absorption rate constant of sulfamethoxazole

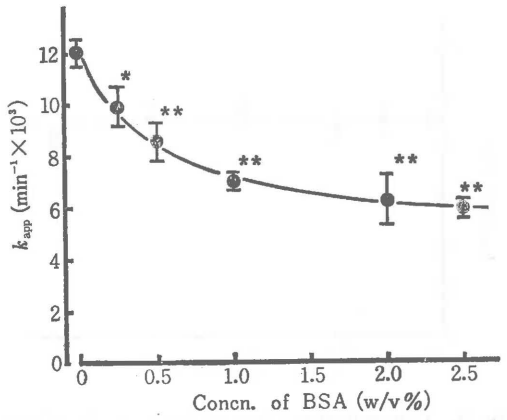

Fig. 1. Concentration Effect of BSA on the Absorption Rate Constant of Sulfamethoxazole in the Rat at pH 6.5
Initial concn. of sulfamethoxazole : 1 mM
Each value represents the mean±S.E. for four to ten experiments.
* $p<0.05$ ** $p<0.01$

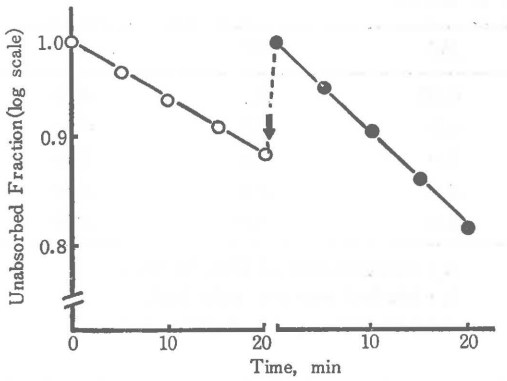

Fig. 2. Logarithmic Plot of Unabsorbed Fraction of Sulfamethoxazole with or without BSA in the Same Rat
Arrow indicates the replacement of perfusion solution containing BSA to those without BSA.
—○— : with BSA (1.25 w/v%)
—⊗— : without BSA

without BSA. As shown in Fig. 2, which represents the typical plot, the decreased rate of absorption with BSA was recovered near to the value for control rat after replacement with perfusion solution without BSA. With respect to the absorption rate constant of sulfanilamide, the decreasing effect of BSA was not observed as shown in Fig. 3.

Binding of Sulfonamides to Bovine Serum Albumin

It is well known that sulfonamides bind to

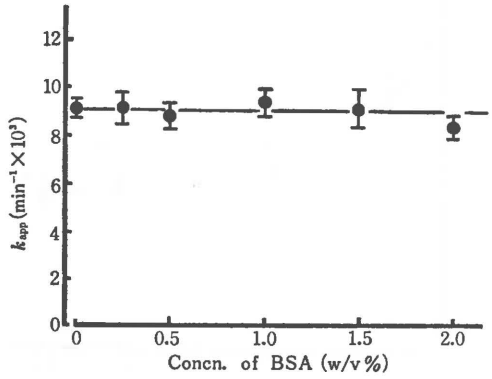

Fig. 3. Concentration Effect of BSA on the Absorption Rate Constant of Sulfanilamide in the Rat at pH 6.5
Initial concn. of sulfanilamide : 1 mM
Each value represents the mean±S. E. for three to eight experiments.

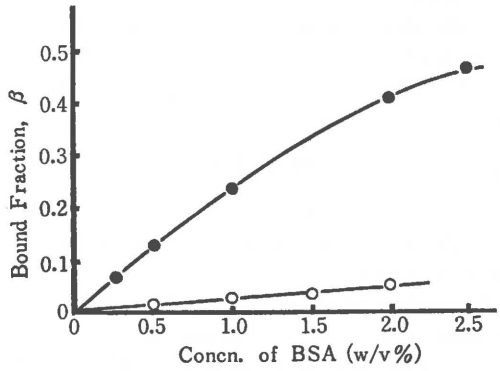

Fig. 4. Concentration Effect of BSA on Bound Fraction of Sulfonamides, β, at 37°C, pH 6.5
—●— : sulfamethoxazole
—○— : sulfanilamide
Total concn. of sulfonamide is 1 mM.

Table II. Binding Parameters of Sulfamethoxazole at 37°C, pH 6.5

P_t[a)]	n[b)]	K[c)]
0.25	2.2	4730
0.5	2.1	4610
1.0	2.1	4520
2.0	2.1	3400
2.5	2.0	3150

a) concentration of BSA (w/v%)
b) bindind sites per mole BSA
c) association constant (litter/mole)

plama protein, particularly to albumin[15]. Binding of sulfonamides to BSA were investigated as a model for macromolecule-drug complex employing an equilibrium dialysis method. Number of binding sites, n, and association constant, K, were calculated from Scatchard's equation (Eq. 3). For various concentrations of BSA, calculated numbers of binding sites and association constants were listed in Table II. Number of drug-binding sites was not influenced by the concentration of BSA, but association constant was slightly decreased as the increase in BSA concentration. The calculated bound fractions were plotted against the concentration of BSA at a total sulfonamide concentration of 1 mM (Fig. 4). Sulfamethoxazole was bound to large extent, whereas, sulfanilamide was little bound in the concentration range of BSA studied.

DISCUSSION

The absorption rate constant of sulfamethoxazole was greatly decreased by addition of BSA to the perfusion solution as shown in Fig. 1. But, the decreasing effect of BSA on the absorption rate was diminished after removing BSA from perfusion solution (Fig. 2). The decreasing effect of BSA on the absorption rate constant of sulfanilamide was not observed (Fig. 3). These findings indicate that BSA does not cause a irreversible damage to the rat small intestine and does not influence the permeability of mucosal membrane. The absorption rate constant of sulfamethoxazole decreased as binding of the drug to BSA increased. Thus, the decreased absorption rate constant will be responsible for the binding of the drug to BSA, leading to the decrease in concentration of unbound drug. Since bound sulfonamide was reported to possess no antimicrobial activity[16], bound sulfonamide is thought to be impermeable to biomembrane. Assuming that the bound drug is impermeable to mucosal membrane and only unbound drug is absorbed by intestinal tract, equation 5 is derived.

$$\frac{dD_t}{dt} = -k_0 \cdot D_f = -k_0 \cdot f \cdot D_t \qquad \text{(Eq. 5)}$$

where, f is the unbound fraction and k_0 is absorption rate constant without BSA. Then, apparent absorption rate constant, k_{app}, is expressed as follows:

$$k_{app} = f \cdot k_0 = (1-\beta) \cdot k_0 \qquad \text{(Eq. 6)}$$

Therefore, a linear correlation would be ex-

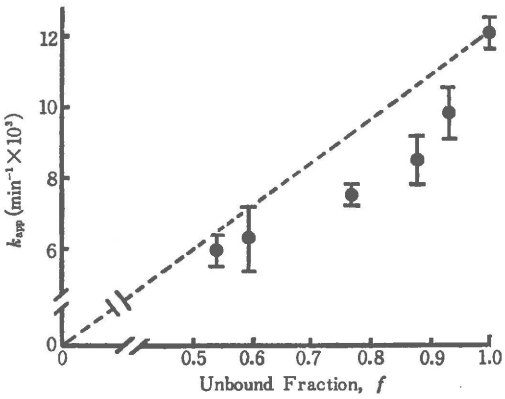

Fig. 5. Relations between the Absorption Rate Constant of Sulfamethoxazole, k_{app}, in the presence or Absence of BSA and Unbound Fraction of Sulfamethoxazole, f

– – – –: theoretical line

Each value represents the mean±S. E. for four to ten experiments.

pected when k_{app} is plotted against unbound fraction, f or $(1-\beta)$. But, as shown in Fig. 5, there is a slight deviation from the theoretical line calculated by assuming that bound drug is unabsorbable. This deviation suggests that the binding of sulfamethoxazole to BSA might be slightly affected in the intestinal tract or on the intestinal mucosal membrane. It is not cleared here, and further investigations are required.

REFERENCES

1) Sonobe, T., Nanbu, N. and Nagai, T.: Pharmacia Review, No. 2. Jap. Pharm. Soc., Tokyo, pp. 75–88, 1979.
2) Hayton, W. L. and Levy, G.: Effect of complex formation on drug absorption. J. Pharm. Sci., 61, 367–371, 1972.
3) Ichibagase, H', Imamura, Y. and Shiozu, K.: Effect of simultaneous administration of drugs on absorption and excretion. XI. Effect of diphenhydramine on sulfisomidine absorption in rabbits. Chem. Pharm. Bull., 29, 887–891, 1981.
4) Tyrer, J. H., Eadie, M. J. and Abelmann, J. M., Haber, E. and Hood, Jr, W. B.: Outbreak of anticonvulsant intoxication in Australian city. Brit. Med. J., 4., 271, 1970.
5) Grisafe, J. A. and Hayton, W. L.: Intestinal absorption of griseofluvin from a triolein digestion mixture in rats. J. Pharm. Sci., 67, 895–899, 1978.
6) Melander, A.: Influence of food on the bioavailability of drugs. Clin. Pharmacokinetics, 3, 337–351, 1978.
7) Greenblatt, D. J., Aleen, M. D., MacLaughlin, D. S., Harmatz, J. S. and Shader, R. I.: Diazepam absorption: Effect of antacid and food. Clin. Pharmacol. Ther., 24, 600–609, 1978.
8) Sugimoto, I.: Studies on complexes XII. Effect of complex formation on drug absorption from alimentary tract. Chem. Pharm. Bull., 18, 524–535, 1970.
9) Singh, P., Guillory, J. K., Sokoloski, T. D., Benet, L. Z. and Bhatia, V. N: Effect of inert tablet ingredients on drug absorption J. Pharm. Sci., 55, 63–68, 1966.
10) Morishita, T., Yata, N. and Kamada, A.: Studies on absorption of drugs. V. Effect of nonionic surfactants on the absorption of drugs. Yakuzaigaku, 31, 187–193, 1971.
11) Kohu, K. W.: Mediation of divalent metal ions in tetracycline to macromolecules. Nature, 191, 1156–1158, 1961.
12) Scatchard, G.: Ann. N. Y. Acad. Sci., 51, 660, 1949.
13) Scatcahrd. G. Scheinberg, I. H. and Armstrong, Jr., S. H.: Physical chemistry of protein solution. IV. The combination of human serum albumin with chloride ion. J. Am. Chem. Soc., 72, 535–540, 1950.
14) Yamazaki, M., Aoki, M., Kamada, A. and Yata, N.: Studies on biological activity of drugs. Yakuzaigaku, 27, 37–40, 1967.
15) Sholtan, W.: Über die Bindung der Langzeitsulfonamide an die Serumeiweiβkörper. Makromolekulare Chem. 54, 24–59, 1962.
16) Anton, A. H.: The relation between the binding of sulfonamides to albumin and their antibacterial efficacy. J' Pharm. Sci., 129, 282–290, 1960.