Chem. Pharm. Bull. 30(3)1077—1080(1982)

Influence of Blood Proteins on Biomedical Analysis. V.[1] Effect of Ethyl Alcohol on Gliclazide-binding with Bovine Serum Albumin

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(Received August 29, 1981)

The effects of ethyl alcohol on the interaction of gliclazide and other sulfonylureas with bovine serum albumin (BSA) were studied by use of an equilibrium dialysis technique. The strongest inhibition of gliclazide-binding with BSA was induced at the drug level which corresponded to the effective dose in the presence of ethyl alcohol (1 mg/ml), resulting in a 2.5-fold higher level of free gliclazide than that of the control. The ratio (D_f/D_t) of free gliclazide concentration to total gliclazide concentration in the medium was constant (about 30%) at concentrations of ethyl alcohol over 0.5 mg/ml. The ratio of D_f/D_t of tolubutamide, glyclazide, acetohexamide and glycopyramide with BSA was raised ($+\Delta 2.1-19.8\%$) in each case, whereas that of chlorpropamide or tolazamide was lowered ($-\Delta 2.9-6.2\%$) by the addition of ethyl alcohol. The addition of ethyl alcohol to the medium reduced the gliclazide-binding ability in the primary binding site on the BSA molecule and reduced the association constant to $2.5\times10^3\,\text{M}^{-1}$ (56.8%) from $4.4\times10^3\,\text{M}^{-1}$ of the control.

In conclusion, it is suggested that one of the reasons for the enhancing effect of ethyl alcohol on the hypoglycemic action of sulfonylurea is the inhibition of binding of the drug with blood protein.

Keywords——ethyl alcohol; sulfonylurea; gliclazide; tolbutamide; chlorpropamide; acetohexamide; glycopyramide; tolbutamide; equilibrium dialysis; bovine serum albumin

Drug-induced dangerous hypoglycemia is well known.[2] In particular, the hypoglycemic action of sulfonylurea, such as tolbutamide, is enhanced by co-administration with ethyl alcohol[2,3] or salicylates.[2,3] It has been reported that the half-life of the drug in the blood of alcoholic test subjects is significantly shorter that in controls[4] and the tolbutamide levels in many organs are enhanced by co-administration with ethyl alcohol in the rat.[5] However, the mechanism of the amplifying effect (or prolonging effect) of ethyl alcohol on the hypoglycemic action of sulfonylurea is still not clearly established.

We considered the amplifying effect of ethyl alcohol from the viewpoint of drug-protein interaction. It is known that the binding of sulfonylureas with circulation proteins, such as albumin or globulin, in body fluid can influence their therapeutic activities.[6] Moreover, it has been accepted that "free sulfonylurea" which is not bound with blood protein exerts the pharmacological effect, hypoglycemic activity.[7] Previously, we reported that gliclazide binds strongly with bovine serum albumin(BSA) or human serum albumin; further, the interactions of gliclazide with native and modified BSA were examined.[8]

In the present paper, we describe the effect of ethyl alcohol on the interaction of gliclazide and five other hypoglycemic agents with BSA. The present binding study was carried out by the use of an equilibrium dialysis technique.[8a]

Materials and Methods

Materials——A pure reference sample of gliclazide was purchased from Dainippon Pharmaceutical Industries Co., Ltd., Japan. Other sulfonylureas, tolbutamide from Hoechst Japan Ltd., chlorpropamide from Taitoh Pfizer Ltd., acetohexamide from Shionogi Pharmaceutical Industries Co., Ltd., glycopyramide

from Kyorin Pharmaceutical Co., Ltd. and tolazamide from Japan Upjohn Ltd., were also used. Bovine serum albumin (fraction V) was obtained from Armour Laboratories U.S.A. and cellophane tubing (Visking Co., 20/32 inch inflated diameter) was used as a dialysis membrane. Ethyl alcohol and other chemicals were products (analytical reagent grade) of Wako Pure Chemical Industries Ltd., Japan.

Equilibrium Dialysis Method——Equilibrium dialysis was performed as described in our previous report.[8a] The concentration of gliclazide was estimated from the optical density at the maximum absorption wavelength (225 nm) of gliclazide at pH 7.4. Avalue of 6.8×10^4 was used for the molecular weight of BSA, and the molecular extinction coefficient of gliclazide in the 1/15 M phosphate buffer (pH 7.4) was taken to be 12.5×10^3. The dialysis studies were done in 1/15 M phosphate buffer (pH 7.4) containing 20 μg/ml of gliclazide with 1×10^3 mM BSA at 37°C.

Calculations——The binding data obtained from the equilibrium dialysis were analyzed according to Scatchard *et al.*[9] and statistical analysis was done by the use of Student's "*t*" test.

Results and Discussion

Fig. 1 shows the relationship between the ratio of D_f/D_t and the gliclazide concentration (D_t) in the medium. The ratio increased dose-dependently with gliclazide concentration up to 0.7 μmol/10 ml and then maintained a constant value of about 35%. When ethyl alcohol (1 mg/ml, effective blood level after drinking) was added to the medium the binding ratio of gliclazide with BSA was lowered, particularly at concentrations (0.03—0.3 μmol/10 ml) corresponding to the blood level produced by an effective dose (oral dose: 80 mg/day),[8b] resulting in a 2.5-fold higher level of free gliclazide as shown in Fig. 1. However, the ratio of D_f/D_t gradually decreased with increase of D_t and the inhibitory effect of ethyl alcohol on the gliclazide-binding with BSA disappeared at around 3 μmol/10 ml of gliclazide (D_t). From this result, the strongest inhibitory effect of ethyl alcohol on the gliclazide-binding with BSA was induced around the drug level corresponding to the effective dose.

The effect of ethyl alcohol concentration on gliclazide-binding with BSA was examined (Fig. 2). The ratio of D_f/D_t increased rapidly from 13% (control) on addition of 0.5 mg/ml of ethyl alcohol to the medium, reaching about 30%, and then remained constant at alcohol concentrations over 0.5 mg/ml until 10 mg/ml.

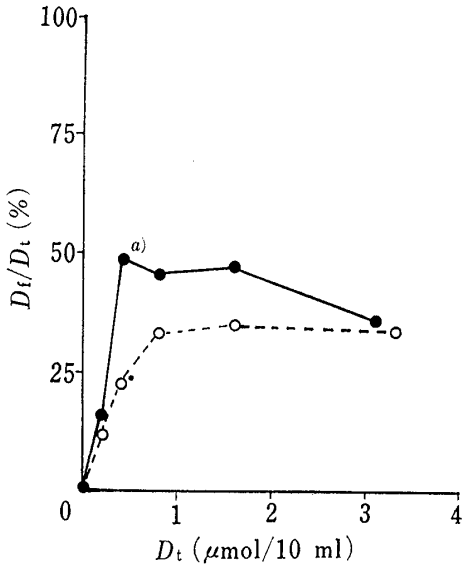

Fig. 1. Effect of Ethyl Alcohol on Gliclazide-binding with BSA

D_t and D_f represent the concentration of gliclazide in the medium(10 ml)and the amount of free gliclazide (unbound with BSA), respectively. Each point represents the mean value of three experiments. ●—●, in the presence of ethyl alcohol (1 mg/ml); ○--○, in the absence of ethyl alcohol (control).
a) $p<0.01$.

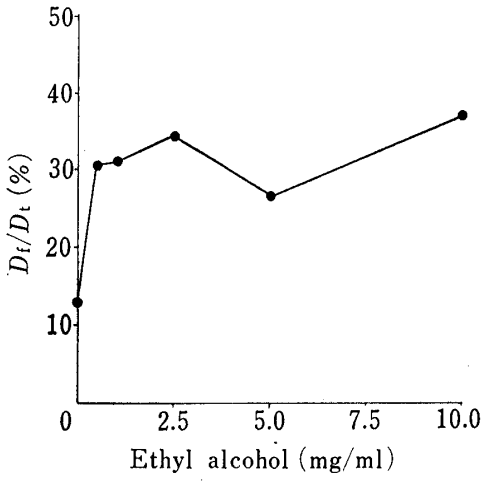

Fig. 2. Dose-relationship between Gliclazide-binding and Ethyl Alcohol Concentration

Each point represents the mean value of three experiments.

The effects of ethyl alcohol (1 mg/ml) on bindings of gliclazide and several other sulfonylureas with BSA, which were tested here by the use of an equilibrium dialysis technique, are summarized in Table I. Each ratio of D_f/D_t in the interactions of tolbutamide, gliclazide, acetohexamide and glycopyramide with BSA increased upon addition of ethyl alcohol, while those of chlorpropamide and tolazamide were lowered, resulting in a decrease of "free sulfonylurea level."

Table I. Inhibitory Effects of Ethyl Alcohol on the Binding of Sulfonylureas with BSA

Sulfonylurea (20 μg/ml)	D_f/D_t (%, mean±SD) Control	D_f/D_t (%, mean±SD) +Ethyl alcohol (1 mg/ml)	Difference (Δ%)	n
Tolbutamide	9.9±0.6	13.6±3.1[b]	+3.7	3
Chlorpropamide	25.1±0.6	18.9±0.6[a]	−6.2	3
Acetohexamide	24.9±0.6	27.0±0.6[b]	+2.1	3
Glycopyramide	13.9±2.8	16.7±2.7	+2.8	3
Tolazamide	39.7±5.8	36.8±1.8	−2.9	3
Gliclazide	12.9±2.9	32.7±4.5[a]	+19.8	3

a) $p<0.01$,
b) $p<0.02$ (*vs.* control).

Scatchard plots for the interaction of gliclazide with BSA in the medium with or without ethyl alcohol (1 mg/ml) are shown in Fig. 3. The Scatchard plot for the interaction of gliclazide with BSA in the absence of ethyl alcohol was hyperbolic, suggesting the existence of two (or more) classes of gliclazide-binding sites on the BSA molecule.[10] The values of K (association constant) and n (equivalents of binding sites) for the interaction of gliclazide with BSA were 0.5 and 4.5 for n_1 and n_2, and 160×10^3 and 4.4×10^3 M^{-1} for K_1 and K_2, respectively. On the other hand, the Scatchard plot for the interaction of gliclazide with BSA in the presence of ethyl alcohol was a single straight line with values of 5.0 for n_2 and 2.5×10^3 M^{-1} for K_2. The addition of ethyl alcohol to the medium destroyed the gliclazide-binding ability in the primary binding site on the BSA molecule and reduced the association constant (K_2) to 2.5×10^3 M^{-1} (56.8%) from 4.4×10^3 M^{-1} for the control.

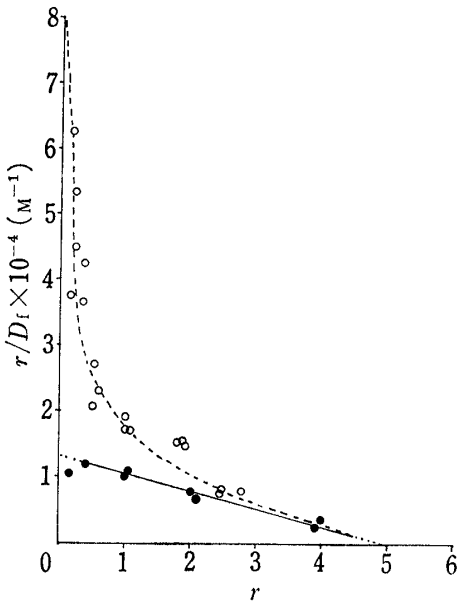

Fig. 3. Effect of Ethyl Alcohol on the Scatchard Plot for the Interaction of Gliclazide with BSA

●—●, in the presence of ethyl alcohol (1 mg/ml); ○--○, in the absence of ethyl alcohol (control). In the control, n_1, n_2, K_1 and K_2 are 0.5, 4.5, 160×10^3 M^{-1} and 4.4×10^3 M^{-1}, respectively. In the presence of ethyl alcohol, n_2 and K_2 are 5.0 and 2.5×10^3 M^{-1}, respectively.

The binding mode for sulfonylurea-binding with BSA has been reported to involve both electrostatic and hydrophobic interactions.[8a,11] The addition of ethyl alcohol, which has a lower dielectric constant (24.2 D at 25°C)[12] than water, to the medium may induce lower ionization of the drug and BSA molecules. In addition, the hydrophobic interaction between the drug and BSA may be decreased by the presence of ethyl alcohol in the medium. The ratio of D_f/D_t may be influenced by the dielectric effect of ethyl alcohol as described above

or by some other physico-chemical interactions among the drug, BSA and ethyl alcohol.

It is known that the hypoglycemic action of sulfonylurea is enhanced (or prolonged) by co-administration with ethyl alcohol.[2,3] Ethyl alcohol itself has no hypoglycemic action except in special circumstances, such as long-term fasting.[13] As described above, it has been reported that ethyl alcohol accelerates the absorption of sulfonylurea from the gut and enhances the drug level in organs,[5] whereas it shortens the half-life of the drug in blood.[4] Thus, we cannot interpret the ethyl alcohol-induced enhancing and prolonging effects on the hypoglycemic action of sulfonylurea simply in terms of modification of absorption or excretion of the drug.

Although the mechanism of the enhancing and prolonging effects of co-administration of ethyl alcohol on the hypoglycemic action of sulfonylurea is not clear, the elevation of "free drug level" due to the ethyl alcohol-induced inhibition of sulfonylurea-blood protein binding appears to be one factor.

Unlike other sulfonylureas, D_f/D_t for chlorpropamide and tolazamide, which can also induce enhancing and prolonging effects on the hypoglycemic action upon co-administration with ethyl alcohol, were lowered by the addition of ethyl alcohol. The lowering of D_f/D_t for those two sulfonylureas may be based on differences of chemical properties, such as higher hydrophobicity, as compared with other sulfonylureas. The characteristic effects of those two sulfonylureas may be predominantly due to actions on other factors, such as drug absorption or excretion, than on drug-blood protein binding.

References and Notes

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