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Role of Acetone Bodies in the Abnormal Pharmacokinetic Behavior of Chlorpropamide in Alloxan Diabetic Rabbits[1,2]

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The effect of acetoacetic acid (AA) or β-hydroxybutyric acid (HBA), each of which is a component of acetone bodies, on the pharmacokinetic behavior of chlorpropamide (CPA) was studied in order to elucidate the reason for the abnormal behavior of CPA under diabetic conditions. The serum level of CPA in the elimination phase showed a rapid and profound fall immediately after intravenous administration of AA. A change of distribution of CPA in the blood was observed in a normal rabbit. However, under diabetic conditions, with high blood levels of acetone bodies, the effect of AA was temporary.

A marked decrease of proximal tubular secretion of CPA was observed under normal conditions after the administration of AA. HBA showed effects similar to those of AA.

A marked increase of CPA in red blood cells was observed in the presence of AA or HBA in normal rabbits. These results coincide with those obtained under diabetic conditions.

It was concluded that the retardation of urinary excretion and the increase in the distribution of CPA into red blood cells in alloxan diabetic rabbits is mainly due to the presence of acetone bodies in the blood.

Keywords——pharmacokinetics in disease state; alloxan diabetics; chlorpropamide; oral antidiabetics; acetone bodies in blood; acetoacetic acid; β-hydroxybutyric acid; distribution into red blood cells; renal clearance

In the preceding paper, the pharmacokinetic features of chlorpropamide (CPA) and sulfadimethoxine (SDM) in alloxan diabetic rabbits were studied and abnormal behavior, such as a decrease in renal clearance and an increase in renal tubular reabsorption under diabetic conditions, was reported.[4] The abnormal behavior of SDM was considered to be mainly attributable to the increased renal reabsorption due to aciduria.[5]

In the present work, in order to elucidate the reason for the abnormal behavior of CPA under diabetic conditions, the effects of acetone bodies in the blood on the abnormal behavior of CPA were studied by administering acetoacetic acid (AA) and β-hydroxybutyric acid (HBA), the main components of acetone bodies, to normal and alloxan diabetic rabbits.

Experimental

Preparation of Alloxan Diabetic Rabbits——Male white rabbits weighing 2.5—3.0 kg were used. The alloxan diabetic rabbits was obtained as reported previously.[6]

Analytical Method——Determinations of Glucose and acetone bodies in the blood and of total protein and albumin in the serum were carried out as described in the previous report.[6] Chlorpropamide (CPA)

1) The report forms part V of "Pharmacokinetic Behavior in the Disease State." Part IV: T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3378 (1978).
2) A part of this work was presented in the 9th symposium on "Drug Metabolism and Action" sponsored by the Pharmaceutical Society of Japan, Kumamoto, November 1977.
3) Location: *133-1, Yamada-Kami, Suita, Osaka, 565, Japan.*
4) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3353 (1978).
5) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3378 (1978).
6) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2238 (1978).

in the blood or serum was determined by high pressure liquid chromatography (HPLC).[4] Serum protein binding of CPA was determined as reported in the previous paper.[7]

Effect of Acetoacetic Acid and β-Hydroxybutyric Acid on the Distribution of CPA in Blood——Animals were first given an intravenous dose of 50 μmol/kg of CPA. A period of 5 hr or more was then allowed for the drug to distribute throughout the body and to enter its subsequent β-phase elimination. At the initial stage of the β-phase, three blood samples were taken at appropriate intervals to determine the elimination rate. Immediately after the third collection of urine, the lithium salt of AA or sodium salt of HBA was intravenously administered into an ear vein at a dose of 10 mg acid %/kg or 20 mg acid %/kg, respectively. Twenty min after the administration of AA or HBA, urine and blood samples were similarly collected three times. After collection of the urine sample, a 0.1 M solution of probenecid was intravenously administered at a dose of 15 μmol/kg. Probenecid was used as an inhibitor of renal tubular secretion of CPA. Urine and blood samples were similarly collected three times starting at twenty min after the administration of probenecid. The concentrations of CPA in the serum and urine were assayed by HPLC and the amount of CPA excreted during each 10 min period was calculated. Part of each serum sample taken for the assay of CPA was used for assay of the concentration of free CPA (not bound to serum protein) and also for the assay of albumin concentration by the methods described in the previous paper.[4]

Clearance of CPA was obtained employing eqs. 1 and 2.

$$Cl_T = (UV)_{10}/C_p \qquad \text{eq. 1}$$

$$Cl_f = (UV)_{10}/C_f \qquad \text{eq. 2}$$

where Cl_T and Cl_f are the renal clearances of CPA in terms of the total concentration and the free concentration of CPA in serum, respectively. $(UV)_{10}$ is the amount of CPA excreted in the urine in 10 min, C_p is the concentration of total CPA in serum, and C_f is the concentration of free CPA (not protein-bound).

To measure the glomerular filtration rate for each rabbit, inulin clearance was routinely determined as described in the previous report.[4]

Distribution of CPA into Red Blood Cells——1) *In Vivo* Study: Following an intravenous administration of CPA at a dose of 100 μmol/kg, one-ml blood samples were taken at appropriate intervals. Aliquots of each sample were used to determine hematocrit, and the concentrations of CPA in the whole blood and serum were obtained. The fraction of CPA distributed into red blood cells was calculated in terms of the apparent distribution ratio (%) as follows:

Apparent distribution ratio (%)

$$\text{Apparent distribution ratio (\%)} = [B-(1-H_t)P] \times 100/B \qquad \text{eq. 3}$$

where P and B are the concentrations of CPA in the serum and whole blood, respectively. H_t represents the value of hematocrit.

2) *In Vitro* Study: Solutions of CPA at various concentrations in blood were prepared by dissolving CPA powder in whole blood freshly taken from rabbits. The blood samples were shaken vertically for 30 min at 30 strokes/min in a water bath at 37°. After reaching equilibrium, the concentration of CPA in the serum and the value of hematocrit were routinely determined. The apparent distribution ratio of CPA in the blood was calculated using Eq. 3.

3) Erythrocyte Suspension: Plasma from heparinized rabbit blood was separated and the erythrocytes were washed three times in 5 times their volume of physiological saline. The cells were resuspended in pH 7.4 phosphate buffer (1/15 M) to give a hematocrit value of 0.36—0.40. The isotonicity was adjusted with NaCl. The distribution of CPA into red blood cells in the suspension was determined as in the *in vitro* study.

Results and Discussion

Effects of AA and HBA on the Concentration of CPA in Serum and Whole Blood at the Postdistribution Phase

The time courses of serum and whole blood levels of CPA before and after administration of AA to a rabbit under normal and diabetic conditions are presented in Fig. 1 (a) and (b). Immediately after the injection of AA, there was a rapid and profound fall in the levels of CPA in the serum and whole blood of the normal rabbit; the fall was complete within 20 min. A rapid change of the distribution of CPA between red blood cells and serum was observed (Fig. 1. (a)).

7) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2058 (1978).

In a diabetic condition, the same rabbit showed a temporary decrease in the serum and whole blood levels of CPA on the injection of AA (Fig. 1 (b)): the whole blood levels of CPA were always higher than those of the serum even after the injection of AA.

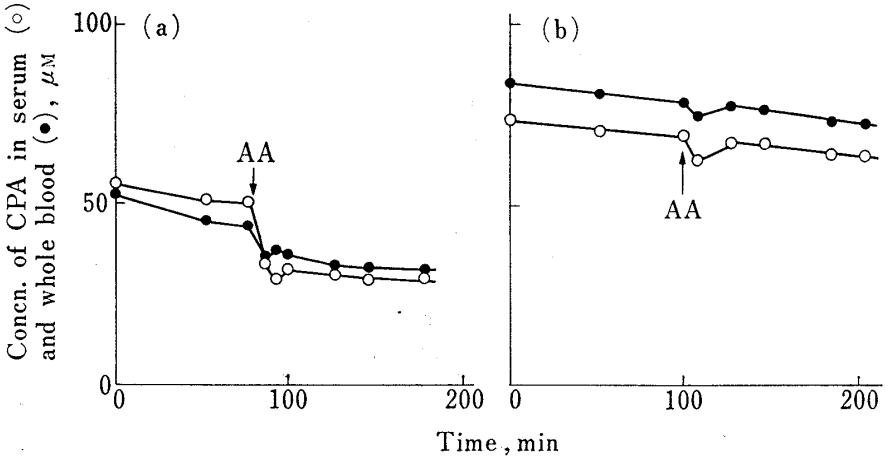

Fig. 1. Effect of Acetoacetic Acid on the Concentration of CPA in the Serum and Whole Blood at the Elimination Phase after Intravenous Administration of CPA

(a): normal. (b): diabetic.

The marked decrease in serum and whole blood levels of CPA in normal rabbits after the administration of AA suggests that a possible redistributional process from blood to tissue should be considered. As reported in the previous paper,[4] in the diabetic condition, in which high blood levels of acetone bodies are found, the distribution volume of the tissue compartment of CPA increased significantly, though that of the central compartment did not change. The finding that the injection of AA into normal rabbits resulted in a marked decrease in the serum level of CPA, below that of whole blood, is closely correlated with the results for rabbits under diabetic conditions.

Elimination rate constants of CPA from serum before and after administration of AA (β and β', respectively) are summarized in Table I.

Table I. Elimination Rate Constants of CPA from the Serum before and after Administration of AA to Normal, Alloxan Diabetic and Insulin-treated Rabbits

	β	Ac	β'	Ac′	Weeks
Normal-1	1.81	0.72	0.85	3.14	0
Normal-2	1.69	0.68	1.06	3.18	1
AD -1	1.02	2.79	0.74	3.42	4
AD -2	1.13	3.38	0.80	3.71	5
AD-In-D	1.88	0.48	0.78	3.07	6
AD-In-W	1.78	0.69	0.96	2.87	10
AD -3	0.48	3.98	0.43	4.72	13
AD -4	0.53	4.86	0.51	5.16	15

β, β': $\times 10^3$ min^{-1}, β is the elimination rate constant from the serum before administration of AA,. β' is the elimination rate constant from the serum after administration of AA. Ac, Ac′: mg/100 ml, Ac and Ac′ are the acetone bodies in whole blood before and after administration of AA, respectively. AD indicates the alloxan diabetic condition, AD-In-D and AD-In-W refer to insulin treatment of alloxan diabetic animals (D, 3 days; W, 4 weeks).

Generally, a decrease in the rate constant was observed after the administration of AA. The extent of the decrease depended on the diabetes picture. This is equivocal evidence

that the extent of decrease in the elimination rate of CPA on administration of AA is dependent on the initial blood concentration of acetone bodies.

These findings suggest that acetone bodies in the blood due to diabetes influence the elimination of CPA from the serum. The decrease in the elimination rate on injection of AA should be mainly due to the retardation of renal excretion of CPA, since the drug is not metabolized in rabbits.

Similar results were obtained for the administration of HBA.

Effect of AA and HBA on the Renal Clearance of CPA

To clarify the retardation of renal excretion of CPA by acetone bodies, the clearance ratio, CR_f, *i. e.*, the ratio of renal clearance of CPA (Cl_f) to the inulin clearance (Cl_{inulin}), was determined before and after administration of AA to a rabbit under normal and diabetic conditions. A marked decrease in CR_f was observed in the normal rabbit (Fig. 2). It can be concluded that the renal excretion of CPA is retarded by the administration of AA.

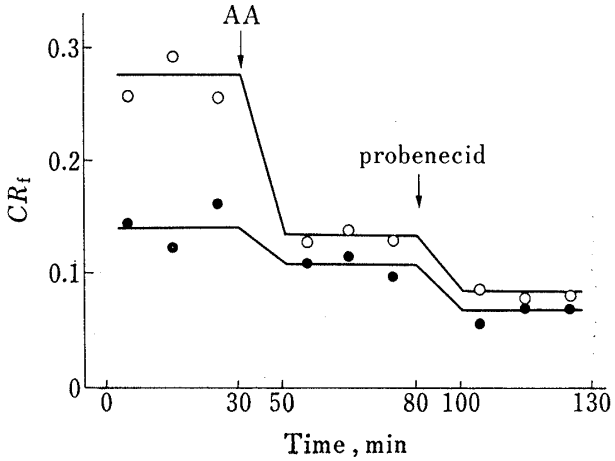

Fig. 2. Effect of Acetoacetic Acid on the Clearance Ratio (CR_f) of CPA

○: normal, ●: diabetic.

In the diabetic condition, a decrease in CR_f on administration of AA was also observed, but was not large in comparison with that of the normal rabbit.

As reported already,[4,6] acetone bodies in blood increase in diabetes. AA is one of the main components of acetone bodies. Thus, a considerable amount of AA is already present in the blood in the diabetic condition used here, and the value of CR_f is already decreased considerably from the normal level. The administration of AA thus causes an insignificant further decrease in CR_f.

An intravenous administration of probenecid resulted in a further decrease in the value of CR_f in rabbits under normal and diabetic conditions.

Table II. Effects of Acetoacetic Acid on Inulin Clearance (GFR·P_f), Renal Tubular Secretion (S) and Reabsorption (R) of CPA in Normal and Alloxan Diabetic Rabbits

		GFR·P_f (ml/min)	S (μmol/min)	R (%)
Normal-1		7.9±0.5	210.5±25.6	91.3±0.9
	AA	8.0±0.4	51.9± 3.4	
Normal-2		8.0±0.3	189.4±41.3	90.7±1.0
	AA	7.9±0.3	45.2± 3.0	
AD-1		8.0±0.6	98.9±19.1	94.2±1.1
	AA	8.3±0.3	54.0±10.0	
AD-2		8.2±0.4	54.3±10.3	92.1±0.8
	AA	8.1±0.5	37.8±15.9	
AD-In-D		8.4±0.4	122.7±20.9	88.5±1.2
	AA	8.4±0.5	36.2± 1.8	
AD-In-W		8.4±0.4	167.9±17.1	89.0±0.8
	AA	8.2±0.2	52.6±12.9	

Symbols: as in Table I.

As shown in Table II, inulin clearance (GFR·P_f) was not affected by the administration of AA to a normal rabbit. Similar results were obtained for the diabetic rabbit. Relative proximal secretion (S) was calculated on the basis of GFR·P_f values following the method described in the previous paper[4] (Table II).

The proximal tubular secretion of CPA showed a marked decrease on administration of AA to a normal rabbit.

The administration of HBA had a similar effect on the renal clearance of CPA.

Effects of AA and HBA on the Distribution of CPA into Red Blood Cells

The distribution of CPA into red blood cells *in vivo* will be influenced by the plasma protein binding of CPA. However, the presence of AA or HBA in the blood at a concentration of 10 mg acid/dl or 20 mg acid/dl, respectively, or more did not affect the protein binding of CPA in rabbits under normal and diabetic conditions.

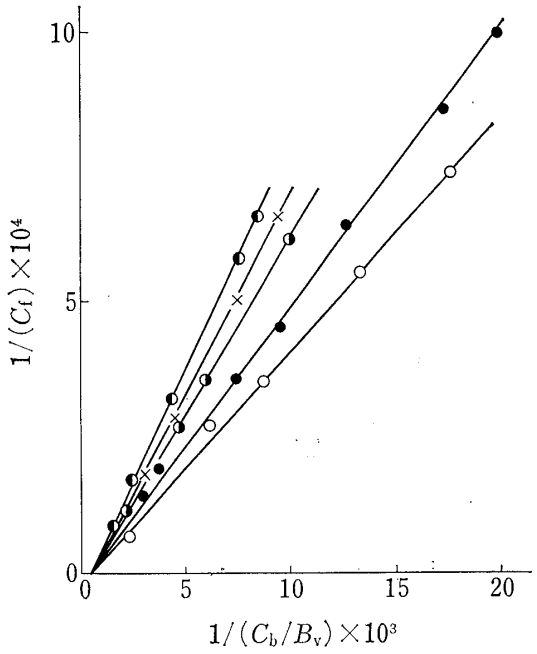

Fig. 3. Langmuir Plots for the Distribution of CPA into Red Blood Cells

●: normal,
◑: AD-1, (AD: alloxan diabetic),
×: AD-2,
◐: AD-3,
○: diabetic rabbit given insulin treatment.

In order to estimate the distribution of CPA into red blood cells *in vivo*, blood samples from rabbits under normal and diabetic conditions were collected at appropriate time before and after administration of AA and the concentrations of CPA in whole blood and in red blood cells were assayed. The results were plotted following Langmuir's equation (Fig. 3). Instead of the terms r in this equation, the amount of CPA distributed into red blood cells (μ mol) in one ml of red blood cells, C_b/B_v, was used. Linear plots were obtained for rabbits under normal and diabetic conditions. These results suggest that the distribution of CPA into red blood cells follows Langmuir's adsorption isotherms, as for the protein binding of CPA.

From the slope and intercept of the line, the constants n and K can be calculated. In the present experiments, K is the degree of distribution of CPA into red blood cells.

As shown in Table III, the values of n remained almost constant, independent of the disease condition and the concentration of acetone bodies in the blood. However, the value of K increased with increase in the concentration of acetone bodies.

Table III. The Degree of Distribution of CPA into Red Blood Cells *in Vivo* in Normal and Diabetic Rabbits

	Acetone bodies in blood (mg/100 ml)	K ($\times10^3$ M^{-1})	n (M)
Normal	0.71±0.14	3.38±0.44	1.57±0.16
AD-1	2.15±0.24	4.51±0.12	1.52±0.13
AD-2	3.50±0.32	5.22±0.07	1.48±0.19
AD-3	4.45±0.18	6.10±0.24	1.51±0.13
AD-In-D	0.35±0.03	2.69±0.32	1.54±0.14
AD-In-W	0.83±0.11	2.98±0.17	1.52±0.11

Symbols: see Table I.

To clarify the effect of acetone bodies on the distribution of CPA into red blood cells, the effect of AA on the distribution of CPA was studied *in vitro* using blood freshly collected from normal rabbits (Table IV). The values of n remained almost constant while K increased with increase in the concentration of AA added.

Table IV. Effect of Acetoacetic Acid on the Distribution of CPA into Red Blood Cells *in Vitro*

Acetoacetic acid (mg/100 ml)	K ($\times 10^3$ M^{-1})	n (M)
0.0	2.86±0.21	1.62±0.12
1.0	3.56±0.25	1.63±0.10
2.5	4.84±0.32	1.53±0.13
5.0	5.32±0.24	1.57±0.10
10.0	6.48±0.29	1.49±0.12

In a physiological saline suspension of red blood cells, a similar concentration-dependent increase in the value of K was obtained (Table V).

Table V. Effect of Acetoacetic Acid on the Distribution of CPA into Red Blood Cells *in Vitro* in the Presence of Ca^{2+}

Acetoacetic acid (mg/100 ml)	Ca^{2+} (mg/100 ml)	K ($\times 10^3$ M^{-1})	n (M)
0.0	10.0	2.75±0.20	1.58±0.18
	30.0	2.80±0.18	1.54±0.11
1.0	10.0	3.01±0.21	1.61±0.14
	30.0	2.85±0.19	1.58±0.13
2.5	10.0	3.55±0.14	1.58±0.14
	30.0	3.04±0.30	1.54±0.11
5.0	10.0	4.22±0.17	1.58±0.08
	30.0	3.25±0.08	1.64±0.13
10.0	10.0	5.01±0.18	1.50±0.14
	30.0	3.89±0.21	1.52±0.13

These findings indicate that the presence of acetone bodies in blood facilitates the distribution of CPA into red blood cells. The presence of HBA in the blood had an effect similar to that of AA on the distribution of CPA into red blood cells.

The effects of AA and HBA can be explained on the basis of a possible modification of the cell membrane by AA and HBA, resulting in an increase in the permeation of CPA through the cell membrane. Solubility and spectroscopic studies did not suggest any interaction between CPA and AA or HBA in the range of concentration used in this study.

AA and HBA have a chelating action on Ca^{2+}. The chelation of Ca^{2+} at the red blood cell membrane is considered to be a possible effect of AA and HBA facilitating the distribution of CPA to red blood cells. Ethylenediamine tetraacetic acid (EDTA), a chelating agent, promotes the intestinal absorption of many drugs due to the removal of Ca^{2+} from the intraluminal surface of the intestine. The effect is marked for hydrophilic compounds but not for lipid-soluble ones.[8] To study the possible effects of AA and HBA on the red cell

8) E. Windsor and G.E. Cronheim, *Nature* (London), **190**, 236 (1961); L.S. Schanker and J.M. Peterson, *Biochem. Pharmacol.*, **8**, 421 (1961); C.S. Tidball and K.K. Peterson, *Physiologist*, **4**, 121 (1961); H. Kunze, G. Rehbock, and W. Vogt, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **273**, 331 (1972).

membrane, the effect of Ca^{2+} on the distribution of CPA into red blood cells was studied in the presence or absence of AA in the red blood cell suspension.

As shown in Table VI, addition of Ca^{2+} alone up to 30 mg/dl to the red cell suspension failed to affect the values of K (compare with Table V). However, in the presence of AA, addition of Ca^{2+} had an inhibitory effect on the action of AA to enhance the distribution of CPA into red blood cells.

Table VI. Effect of Acetoacetic Acid on the Distribution of CPA into a Red Blood Cell Suspension in the Presence and Absence of Ca^{2+}

Acetoacetic acid (mg/100 ml)	Ca^{2+} (mg/100 ml)	K ($\times 10^3$ M^{-1})	n (M)
0.0	0.0	3.05±0.08	1.53±0.03
	10.0	2.98±0.11	1.52±0.14
	30.0	2.99±0.09	1.60±0.11
1.0	0.0	3.67±0.27	1.54±0.05
	10.0	3.04±0.12	1.50±0.11
	30.0	2.75±0.28	1.52±0.08
2.5	0.0	4.71±0.13	1.51±0.15
	10.0	3.72±0.24	1.52±0.16
	30.0	2.95±0.21	1.57±0.12
5.0	0.0	5.88±0.17	1.42±0.13
	10.0	4.68±0.13	1.48±0.13
	30.0	3.69±0.26	1.59±0.20

Thus, it can be concluded that the retardation of urinary excretion and the increase in the distribution of CPA into red blood cells in alloxan diabetic rabbits is mainly due to the presence of acetone bodies in the blood.