EFFECT OF ACETAZOLAMIDE ON BARBITURATE-INDUCED SLEEPING TIME IN MICE. III.* PHARMACOKINETICS OF SERUM ELIMINATION AND BRAIN DISTRIBUTION

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Effects of acetazolamide (AZA) on the serum elimination and brain distribution of barbital (BA), phenobarbital (PHB), pentobarbital (PEB) and hexobarbital (HB) were studied in mice.

When the barbiturates were administered intraperitoneally to mice, the pretreatment of AZA reduced the serum BA and PHB levels, and significantly increased these brain levels. While relatively small and no effects of AZA were observed for the PEB and HB levels, respectively.

After the intravenous administration, the serum elimination of these barbiturates were described by the two compartment model. Although the pretreatment of AZA tended to increase the volumes of central compartment and decrease the elimination rate constants for BA, PHB and PEB, the elevated brain levels of the barbiturates could not be explained as the simulated peripheral concentrations.

However, it appeared that the prolongation effect of AZA on the BA, PHB and PEB sleeps in mice was associated with the elevated brain barbiturate levels with AZA.

Keywords—acetazolamide; barbital; phenobarbital; pentobarbital; hexobarbital; brain; serum; concentration; drug interaction; pharmacokinetics

INTRODUCTION

Previous works in this series^{1,2)} have shown that the sleeping times induced with several barbiturates were significantly prolonged by the pretreatment of acetazolamide (AZA) in mice. Since the brain barbiturate concentrations in the pretreated mice were almost the same as that in the control mice at the moment of awakening, it was suggested that this effect of AZA on the sleeps was not associated with the modification of sensitivities for the sleep but related to the elevation of the brain barbiturate levels. However, there was some exception like hexobarbital, which sleeping time was rather shortened by AZA treatment.¹⁾ Thus, to elucidate the effect of AZA on the sleeps as the modification of the

brain barbiturate levels, it was necessary to compare the time course of the brain barbiturates in the control mice with that in the AZA treated mice.

For this purpose four barbiturates were chosen in this study; that is, barbital (BA), phenobarbital (PHB) and pentobarbital (PEB) as the examples in which sleeps were prolonged with AZA,^{1,2)} and hexobarbital (HB) as the example in which sleep was shortened with AZA.¹⁾ The effect of AZA pretreatment on the time courses of the barbiturate concentrations in the brain as well as in the serum were studied after the intraperitoneal administration in mice.

Furthermore, the AZA effect on the serum elimination of the barbiturates after the

* Part II: see ref. 2.

intravenous administration was analyzed pharmacokinetically to characterize the interaction between AZA and the barbiturates.

MATERIALS AND METHODS

Animals — Male ddY albino mice weighing 25–30 g were used. They were kept in a room maintained on a 12 h light-dark schedule at 22–24°C. Food and water were provided *ad libitum*.

Chemicals — Sodium AZA (Diamox® Parenteral, Lederle Japan Ltd.) and HB (Cyclopan®, Teikokukagakusangyo Co., Ltd.) were purchased from commercial sources. Sodium salts of BA, PHB and PEB were same as previously reported.^{1,2)} All other chemicals used were of analytical grade.

Animal Experiments — All the methods for preparation of drug solutions and procedures for the *i.p.* administration experiments were as described previously.^{1,2)} In the *i.v.* administration experiments, sodium AZA was dissolved in distilled water and administered to the mice at the dose of 100 mg/kg body weight (*i.p.*) 30 min before the barbiturate injection. Sodium salts of BA, PHB and PEB were dissolved in saline solution. HB was dissolved in saline solution with equimolar NaOH. The barbiturates were injected into the mice through the tail vein at the dose of 50, 60, 60 and 71 mg/5 ml/kg body weight as sodium salts of BA, PHB, PEB and HB, respectively. At each given time, 3–6 mice were killed to obtain the blood and brain samples.

Determination of Barbiturate Concentrations in Serum and Brain — After sacrificing the mice, BA, PEB and HB in the serum and brain were extracted with chloroform and then determined by the gas chromatographic methods.^{1,2)} PHB in the serum and brain was measured by the high performance liquid chromatographic method.³⁾

Binding Experiments — The binding of barbiturates to the serum protein was measured with an ultrafiltration apparatus (MPS-1 with YMT membrane, Amicon Co.). A half milliliter of the pooled mouse serum containing 20 µg/ml of barbiturates was centrifuged to obtain about 0.2 ml

of ultrafiltrate. When the effect of AZA on the binding was examined, AZA was spiked into the serum at a concentration of 100 µg/ml. The adsorption of barbiturates to the membrane was negligible in this experimental condition.

Pharmacokinetic Analysis — The serum elimination of barbiturates after *i.v.* administration was analyzed by the computer program, AUTOAN⁴⁾ which accepts raw blood level data, strips the data, chooses the appropriate model, then goes on to yield a final least squares fit of the data to the chosen model. Minicomputer system, MELCOM 70/30 (Mitsubishi Electric Co., Ltd.) was used for this purpose.

RESULTS

1. Effect of AZA on the Serum and Brain Concentration of Barbiturates administered intraperitoneally

To relate the time course of the brain barbiturate levels to the sleeping time data reported previously,^{1,2)} the mice were given the same pretreatment with AZA (25 mg/kg, *i.p.* 30 min prior to the barbiturate) and then the same doses of barbiturates (*i.p.*) as in the previous papers.

The effect of AZA on the time course of the BA levels in the brain and serum are summarized in Fig. 1a. The pretreatment with AZA tended to decrease the serum level, but on the contrary, significantly increased the brain level of BA. As a consequence, the brain/serum concentration ratio (*B/S*) of BA was significantly elevated by the AZA pretreatment.

As shown in Fig. 1b, essentially the same trend was observed for PHB. Increased *B/S* of PHB by the AZA pretreatment was more obvious than that of BA.

Although the AZA pretreatment increased a little the brain level of PEB (but not significantly), it did not affect the serum PEB level and also the *B/S* (Fig. 2a).

In the case of HB (Fig. 2b), actually no effect of AZA was found not only on the serum level but also on the brain level.

2. Effect of AZA on the Serum and Brain Concentration of Barbiturates administered intravenously

In order to examine the effect of AZA on the

FIG. 1. Time Courses of Serum and Brain Concentration, and Brain/Serum Concentration Ratio of Barbit(a) and Phenobarbital(b) after i.p. Administration in Mice

AZA (25 mg/kg, i.p.) was administered 30 min before i.p. administration of BA (200 mg/kg as sodium salt) or PHB (120 mg/kg as sodium salt). Each point represents mean $\pm$ S.E. of 3–4 experiments. a) Significantly different from control at a p value of 0.05 or less (t -test).

Control (—○—), AZA treated (---●---).

FIG. 2. Time Courses of Serum and Brain Concentration, and Brain/Serum Concentration Ratio of Pentobarbital(a) and Hexobarbital(b) after i.p. Administration in Mice

AZA (25 mg/kg, i.p.) was administered 30 min before i.p. administration of PEB (40 mg/kg as sodium salt) or HB (70 mg/kg as sodium salt). Each point represents mean $\pm$ S.E. of 4-6 experiments.

a) Significantly different from control at a *p* value of 0.05 or less (*t*-test).

Control (—○—), AZA treated (---●---).

FIG. 3. Time Courses of Serum and Brain Concentration, and Brain/Serum Concentration Ratio of Barbitol(a) and Phenobarbital(b) after i.v. Administration in Mice

AZA (100 mg/kg, i.p.) was administered 30 min before i.v. administration of BA (50 mg/kg as sodium salt) or PHB (60 mg/kg as sodium salt). Each point represents mean $\pm$ S.E. of 3–6 experiments. a) Significantly different from control at a p value of 0.05 or less (t -test).

Observed concentration: control ($\circ$), AZA treated ($\bullet$), calculated time course for C_1 (i), C_2 (ii) and C_2/C_1 (iii): control (—), AZA treated (---).

FIG. 4. Time Courses of Serum and Brain Concentration, and Brain/Serum Concentration Ratio of Penobarbital(a) and Hexobarbital(b) after i.v. Administration in Mice

AZA (100 mg/kg, i.p.) was administered 30 min before i.v. administration of PEB (60 mg/kg as sodium salt) or HB (71 mg/kg as sodium salt). Each point represents mean $\pm$ S.E. of 3–6 experiments.

a) Significantly different from control at a p value of 0.05 (t -test).

Observed concentration: control ($\circ$), AZA treated ($\bullet$), calculated time course for C_1 (i), C_2 (ii) and C_2/C_1 (iii): control (—), AZA treated (---).

serum elimination pharmacokinetics of barbiturates, the barbiturates were intravenously injected into mice 30 min after the administration of AZA (100 mg/kg, *i.p.*).

The time course of BA concentration in the serum, as well as that in brain and the B/S are shown in Fig. 3a. As seen in the case of the *i.p.* administration, the serum concentrations of BA with AZA were lower than those in control. Correspondingly, the BA brain concentrations, hence B/S too, with AZA were significantly higher than those in control.

According to the pharmacokinetic analysis, the serum eliminations of BA, both with and without AZA, were able to be described well by the 2-compartment open model in Table I. The best fitted lines for the concentration in central compartment (C_1) are depicted in Fig. 3a-i and the parameters are listed in Table I together with

those for other barbiturates.

In comparison with the control, the AZA pretreatment seemed to decrease the initial serum BA level (α phase) and then delay the BA elimination (β phase). This associated with the pharmacokinetic parameters (slight increase in the V_1 and decrease in the k_{el} in Table I).

To know the effect of AZA on the peripheral concentration (C_2) of BA, C_2 were calculated on the basis that $V_2 = V_1 \cdot k_{12}/k_{21}$. The calculated C_2 and also C_2/C_1 lines for BA are depicted in Fig. 3a-ii and iii to compare with the brain concentration and B/S , respectively. Although each observed BA time course of brain level and B/S changed similarly to those of C_2 and C_2/C_1 respectively, the observed AZA effect on the brain concentration was not in accord with the calculated AZA effect on the peripheral concentration.

TABLE I. Effect of AZA^{a)} on the Calculated Pharmacokinetic Parameters^{b)} of Barbiturates in Mice

Barbiturates	<i>i.v.</i> dose (mg/kg as Na salt)	Treatment	k_{12}	k_{21}	k_{el}	V_1
BA	50	Control	0.0037	0.0381	0.00311	0.552
		AZA	0.0062	0.0484	0.00250	0.585
PHB	60	Control	0.0816	0.2150	0.00184	0.486
		AZA	0.0281	0.0939	0.00030	0.604
PEB	60	Control	0.0392	0.152	0.0151	0.908
		AZA	0.0388	0.148	0.0107	0.992
HB	71	Control	0.0778	0.407	0.0374	0.689
		AZA	0.0694	0.234	0.0335	0.699

a) AZA (100 mg/kg, *i.p.*) was administered 30 min before *i.v.* administration of each barbiturate.

b) Two compartment open model.

C : concentration of drug in compartment,
 V : volume of distribution (l/kg),
 k : pharmacokinetic rate constant (min^{-1}).

In the case of PHB (Fig. 3b), more appreciable AZA effect was found, though the essential trend was almost the same as that for BA. The AZA effect on the pharmacokinetic parameters was also remarkable, however, calculated peripheral concentration of PHB was rather lowered by the AZA treatment.

As shown in Fig. 4a, the pretreatment with AZA gave the relatively small effect to the serum PEB elimination and resulted in a slightly increased brain distribution of PEB. The calculated C_2 time courses failed to show the AZA effect similar to that observed in the brain levels.

Fig. 4b shows no significant AZA effect on the serum elimination and the brain distribution of HB. The time courses of calculated C_2 differed

from those of observed brain level.

3. Effect of AZA on the Serum Protein Binding of Barbiturates

Table II shows the percent of barbiturates bound to the mouse serum protein at a concentration of 20 $\mu\text{g/ml}$ *in vitro*. Coexisting AZA at a concentration of 100 $\mu\text{g/ml}$, which was approximately maximum after the *i.p.* administration at a dose of 100 mg/kg in mice, has a slight displacing activity for PHB and HB bound to the protein.

DISCUSSION

As reported previously,^{1,2)} the sleeps induced with intraperitoneally administered BA, PHB and PEB were significantly prolonged by the AZA pretreatment in mice.

In this study, the AZA pretreatment more or less tended to decrease the serum barbiturate levels and increase the brain barbiturate levels (Fig. 1, 2a). Even though the increments in brain barbiturate level are relatively small, this may explain the observed prolongation of the sleeping time. In the case of HB, in which sleeping time was shortened by the AZA pretreatment,¹⁾ the brain level was not lowered with AZA (Fig. 2b). Hence, some other effect¹⁾ must be considered in the HB sleep.

When the barbiturates were intravenously administered in mice, the AZA effect similar to that in the *i.p.* administration experiments was

TABLE II. Effect of AZA on Serum Protein Binding of Barbiturates in Mice

Barbiturates (20 $\mu\text{g/ml}$)	% bound ^{a)}	
	Control	With AZA (100 $\mu\text{g/ml}$)
BA	13.1 $\pm$ 1.05	10.3 $\pm$ 0.78
PHB	30.4 $\pm$ 0.50	24.5 $\pm$ 0.52 ^{b)}
PEB	56.1 $\pm$ 0.67	55.5 $\pm$ 0.66
HB	54.6 $\pm$ 1.10	47.4 $\pm$ 0.18 ^{b)}

a) Mean $\pm$ S.E. (n=3).

b) Significantly different from control at a p value of 0.05 (t-test).

TABLE III. Effect of AZA on the Steady State Brain/Serum Concentration Ratio of Barbiturates after *i.v.* Administration in Mice

Barbiturates	B/S ^{a)}		
	Control	With AZA	Increase (%)
BA	0.70 $\pm$ 0.01 (22)	0.75 $\pm$ 0.01 (23) ^{b)}	7.1
PHB	0.71 $\pm$ 0.01 (23)	0.81 $\pm$ 0.01 (23) ^{b)}	14.1
PEB	1.33 $\pm$ 0.02 (21)	1.41 $\pm$ 0.02 (29) ^{b)}	6.0
HB	0.87 $\pm$ 0.02 (16)	0.87 $\pm$ 0.02 (15)	0

a) Figures denote means $\pm$ S.E. with numbers of observations in parentheses. The brain/serum concentration ratio (B/S) was obtained from the data points in Fig. 3–4: for BA, 30–120 min; for PHB, 30–150 min; for PEB, 20–50 min; for HB, 20–60 min.

b) Significantly different from control at a p value of 0.05 or less (t-test).

observed (Fig. 3, 4). Therefore, the absorption process may not associate with the AZA effect in the *i.p.* administration of barbiturates.

AZA seems to affect the serum elimination of BA and PHB in two different ways; that is, the lowering of the initial serum level (α phase) and the decrease in elimination at the β phase. Though the reasons are not known well as yet, this may be related to the increased V_1 and the decreased k_{el} (Table I).

According to Lin *et al.*,⁵⁾ the distribution of some barbiturates, such as BA and PHB, in rats was so slow that the brain was assigned to a part of peripheral compartment. In our study with mice, the time courses of the observed brain levels of barbiturates except for HB might correspond to those of the calculated peripheral concentrations (Fig. 3, 4). However, the simulated AZA effects on the peripheral barbiturate concentrations failed to show the observed effect on the brain levels.

It is well known that the protein binding and lipid solubility of barbiturate affect its distribution between serum and brain tissues.⁶⁾ As shown in Table III, the pretreatment with AZA significantly raised the steady state B/S of the barbiturates, except for HB. The modification of the barbiturate protein binding with AZA seems not to be important for the AZA effect on the B/S of barbiturates. Because, the displacing activity of AZA (Table II) was not consistent with the AZA effect on the B/S .

AZA, which is a potent carbonic anhydrase inhibitor, may acidify the serum⁷⁾ and increase the undissociated fraction of barbiturates in the serum. This may also enhance the distribution of barbiturates into the brain.⁸⁾ In this case, the more acidic the barbiturates are, the greater the effect by the AZA treatment must be. As the pK_a values of the barbiturates⁹⁾ were reported to be 7.41 for PHB, 7.91 for BA, 8.11 for PEB and 8.34 for HB, the degree of the AZA effect on the B/S was consistent with the acidity of the barbiturates.

Another possible mechanism which is concerned with the acidity of the barbiturates is the inhibition of the acid transport system with AZA.

If AZA, like probenecid,¹⁰⁾ inhibits the acid transport system with which barbiturates are transported out of the brain, more acidic barbiturates should have greater affinity to the transport, and hence receive the more considerable AZA effect on B/S .

The lowering of cerebrospinal fluid flow by the AZA treatment¹¹⁾ might contribute little to the change in the B/S , because if this was the case, almost the same extent of increases in the B/S should be found for each barbiturate.

In conclusion, though the mechanism involved in the interaction is still not clear, it is strongly suggested that some AZA effect associated with the acidity of the barbiturates enhances the distribution of barbiturates in the brain, and therefore, potentiates the activity of the barbiturate sleeps such as BA, PHB and PEB. However, shortened sleeping time of HB with AZA cannot be explained on the basis of the assumption mentioned above.

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