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Relationship between Polymorphism and Bioavailability of Amobarbital in the Rabbit[1)]

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The effect of the polymorphic form of amobarbital on its bioavailability was examined by measurement of the dissolution rate and *in vivo* absorption of two forms (Form I and Form II). The dissolution rate of Form II was faster than that of Form I. In *in vivo* absorption experiments using rabbits, two crystal forms of amobarbital were administered orally and the resulting blood level–time curves were compared with the dissolution rates. It appeared that the polymorphic form of the drug appreciably affected the absorption process from the digestive tract.

Hydroxyamobarbital was confirmed to be the main metabolite of amobarbital.

Keywords——amobarbital; polymorph; dissolution rate; hydroxyamobarbital; absorption rate; oral administration; bioavailability

Many drugs are believed to exist in more than one polymorphic crystal form, and differences in physical properties and bioavailability due to polymorphic crystal form or pseudo-polymorphism have been reported in recent years.[2–5)]

Amobarbital has been reported to have two crystal forms,[6–13)] but nothing is known about their physical properties and gastrointestinal absorption. We have therefore examined the solubility and *in vivo* absorption of these two polymorphic forms of amobarbital, to investigate the relationship of crystal form to bioavailability. It has been reported that amobarbital is metabolized to hydroxyamobarbital,[14–16)] and this was confirmed by thin-layer chromatography.

Experimental

Solubility Measurements——1) Preparation of Samples: a) Form I: Commercial amobarbital (JP, Nippon Shinyaku, Kyoto) was sieved to obtain grains with a diameter of 250—297 μm, which were used for the experiments.

b) Form II: Amobarbital JP was dissolved in acetone with warming (80°), filtered while hot, and the filtrate was poured into 400 ml of water. The crystals that precipitated out were collected by filtration, and dried in a vacuum desiccator. After confirming that these crystals were in Form II by X-ray diffraction and differential scanning calorimetry–thermogravimetry (DSC–TG), as described previously,[17)] we sieved the crystals to obtain grains with a diameter of 250—297 μm.

2) Measurement of Dissolution Rate: This was measured by the tape method of Goldberg *et al.*,[18)] with the apparatus described previously.[19)] Distilled water (400 ml) was placed in a beaker at 37°, 40 mg of the sample powder was dispersed on a double adhesive tape (1.8×5 cm), and dissolution was started.

At each time of measurement, 2 ml of the solution was collected with a cotton-plugged pipette, and 2 ml of fresh distilled water at the same temperature was added to the beaker to maintain the volume of the solution constant. The collected solution was diluted to 5 ml with borate buffer, pH 9.5, and the drug was assayed spectrophotometrically at 238 nm.

Determination of Metabolite——1) Synthesis of Hydroxyamobarbital[14)]: A mixture of 1.35 g of amobarbital, 8.0 g of chromic acid, and 75 ml of AcOH in a round-bottomed flask was stirred at 20° for 30 min. The time and temperature are critical. This reaction mixture was poured slowly into 250 ml of Et_2O and the Et_2O solution was washed with a small quantity of H_2O until the color disappeared completely. The Et_2O solution was dried and evaporated to dryness. The pale greenish, oily residue was triturated with a small quantity of Et_2O, which caused it to crystallize. The crude crystals were recrystallized from H_2O as white needles, mp 187—188°, in 20—30% yield. Further confirmation of the identity of the product as hydroxyamobarbital was obtained by checking the presence of the OH band in its IR

spectrum and of a UV absorption maximum at 255 nm in 0.5 N NaOH solution. *Anal.* Calcd. for $C_{11}H_{18}N_2O_4$: C, 54.52; H, 7.49; N, 11.58. Found: C, 54.75; H, 7.40; N, 11.50.

2) Extraction of Amobarbital and Hydroxyamobarbital from Blood:[20] The plasma (3 ml) was basified with 0.7 ml of 1 N NaOH and extracted with 3 ml of Et_2O for 30 sec. The Et_2O layer was discarded, then the plasma was adjusted to pH 6.5—7.5 with 1 N HCl, and extracted twice with 15 ml each of Et_2O for 3 min. The combined Et_2O extract (30 ml) was evaporated to dryness, the residue was dissolved out three times with 0.1 ml each of Et_2O and this Et_2O solution was spotted on a thin-layer chromatographic plate.

3) Thin-layer Chromatography (TLC): The 5723 TLC plate coated with silica gel 60, F_{254} (Merck Japan, Tokyo) was developed with iso-PrOH: BuOH: NH_4OH (9: 9: 2) for 2 hr after saturation for 15 min; the front moved 10 cm. The spots were detected by UV irradiation (254 nm) and by coloring with copper-pyridine reagent.

Measurement of Blood Level after Oral Administration to Rabbits——A group of 5 male rabbits, weighing 3.2—4.2 kg, was fasted overnight, and 300 mg/kg of the drug with a granular diameter of 63—88 μm suspended in 1% CMC solution was given orally through a catheter. Blood samples were collected from the aural vein, *ca.* 2 ml per time, centrifuged, and the plasma was stored in a refrigerator until use.

For the determination of amorbarbital in the plasma, Goldbaum's absorption measurement was simplified.[21] To 1 ml of plasma, 1 ml of 1 N HCl and 10 ml of $CHCl_3$ were added. The mixture was shaken for 20 min, centrifuged, and 6 ml of the $CHCl_3$ layer was shaken with 5 ml of borate buffer (pH 12.0) for 15 min. This mixture was centrifuged and absorption of the borate buffer layer was measured at 238 nm. The recovery was 94.2±2%.

Results and Discussion

Dissolution Rate

From the results of the dissolution experiments on the two crystal forms of amobarbital in water at 37°, values of $W_0^{1/3} - W^{1/3}$ were calculated from the dissolution rate formula for a multigranular system:

$$W_0^{1/3} - W^{1/3} = kt$$

where W_0 is the initial weight of undissolved granules, W is the weight of undissolved granules at time t, and k is the rate constant. The values thus obtained were plotted against time (Fig. 1). The regression equations of the two straight lines are:

Form I: $Y_1 = 0.008X_1 + 0.021$

Form II: $Y_2 = 0.013X_2 + 0.034$

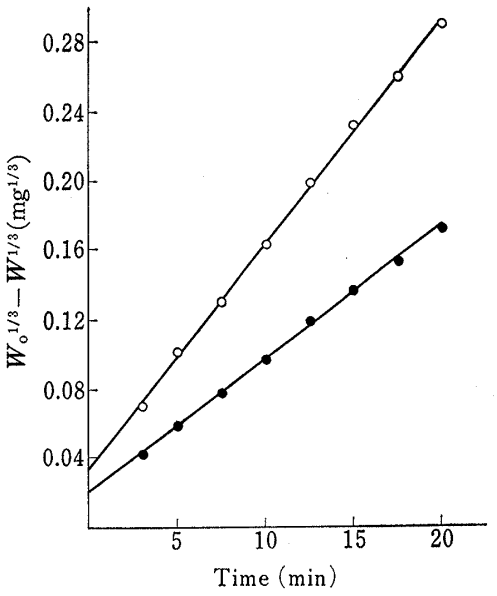

Fig. 1. Dissolution Rates of Polymorphs of Amobarbital

—●— Form I, —○— Form II.

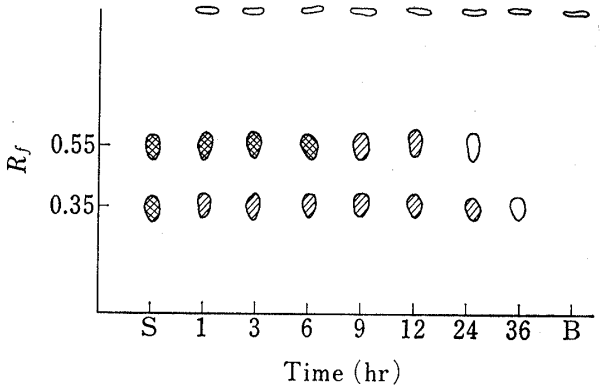

Fig. 2. Thin-layer Chromatogram of Serum Extracts after Oral Administration of 300 mg/kg of Amobarbital to Rabbits

Upper spots (Rf 0.55): unchanged amobarbital,
Lower spots (Rf 0.35): hydroxyamobarbital,
S: control spots of 100 μg each of amobarbital and hydroxyamobarbital,
B: blank.

The slope for Form II is *ca.* 1.6 times greater than that for Form I, indicating that the dissolution rate of Form II is faster than that of Form I.

Detection of Amobarbital and Hydroxyamobarbital

TLC of plasma extracts was carried out (Fig. 2). Although the *Rf* values varied slightly with time, amobarbital at *Rf* 0.55 and hydroxyamobarbital at *Rf* 0.35 were detected. There was an impurity (*Rf* 1.0) in all of the extracts but this was detected even in the extract obtained before administration so that this substance is neither amobarbital nor hydroxyamobarbital.

The metabolite was detected up to 36 hr after administration and the concentration was fairly high. This result indicates that the bulk of amobarbital is converted to the hydroxy compound and excreted. Since the hydroxy compound has an absorption maximum at the same wavelength as amobarbital, the blood level after oral administration can be measured as the total quantity of barbital.

Effect of Polymorphism on Bioavailability

1) **Determination of the Elimination Rate Constant**——A linear plot was obtained after about 6 hr, from which the elimination rate constant (K_E) could be calculated. The relationship between K_E and biological half-life, $t_{1/2}$, is expressed by the following equation:

$$K_E = \frac{0.693}{t_{1/2}}$$

The K_E values of Forms I and II were 3.77×10^{-2} and 3.81×10^{-2} hr^{-1}, respectively. Therefore, differences in the blood levels between Forms I and II are not likely to be due to different elimination rate (Fig. 3).

2) **Determination of the Absorption Rate**——The absorption rate was estimated from the blood level by using Dominguez's formula:[22]

$$\frac{dA}{dt} = Vd\left(\frac{dC_b}{dt} + K_E \cdot C_b\right)$$

where dA/dt is the absorption rate, Vd is the apparent volume of distribution, and dC_b/dt is the rate of change in blood level (C_b) with time, t.

Integration of this formula from time 0 to T, and division by Vd gives

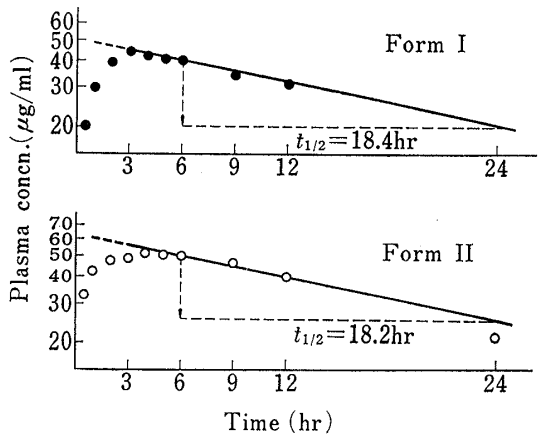

Fig. 3 Semi-logarithmic Plots of the Plasma Concentrations of Polymorphs of Amobarbital

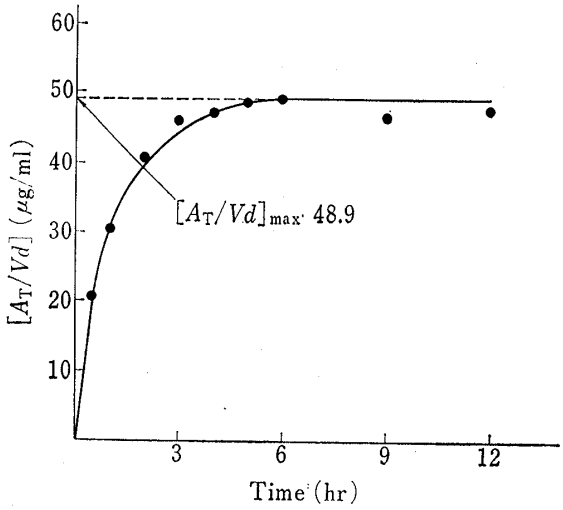

Fig. 4. Plot of A_T/Vd against Time calculated from the Plasma Concentrations of Form I of Amobarbital

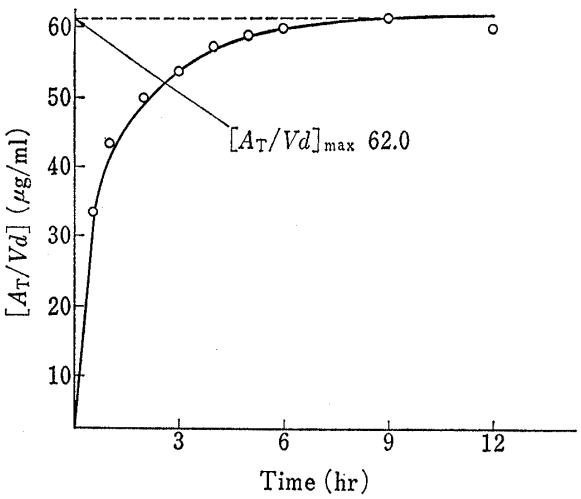

Fig. 5. Plot of A_T/Vd against Time calculated from the Plasma Concentrations of Form II of Amobarbital

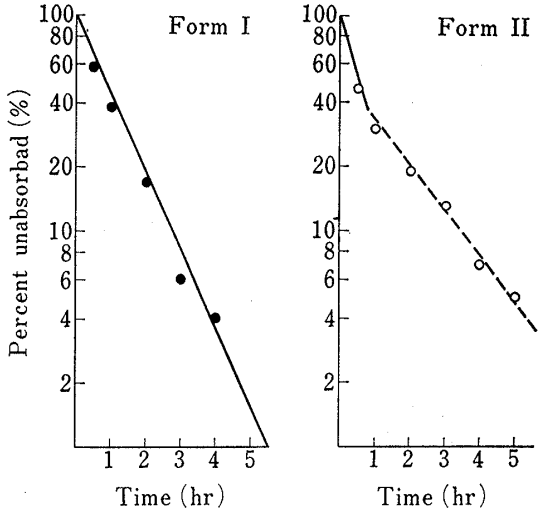

Fig. 6. Apparent First-Order Absorption of the Polymorphs of Amobarbital following Oral Administration

Table I. Calculation of Absorption Parameters from the Plasma Concentrations of Form I of Amobarbital

Time (hr)	C_T (μg/ml)	$[AUC]_{t=0}^{t=T}$ (μg·hr/ml)	$K_E[AUC]_{t=0}^{t=T}$ (μg/ml)	A_T/Vd (μg/ml)	$\frac{(A_T/Vd)_T}{(A_T/Vd)_{max}}$	Percent unabsorbed (%)
0.5	20.35	6.10	0.23	20.58	0.42	58
1	29.74	17.81	0.67	30.41	0.62	38
2	39.33	33.15	1.25	40.58	0.83	17
3	43.75	53.72	2.03	45.78	0.94	6
4	42.03	135.08	5.09	47.12	0.96	4
5	41.59	184.02	6.94	48.53	—	—
6	40.36	225.85	8.51	48.87	—	—
9	33.82	340.98	12.85	46.67	—	—
12	31.17	438.54	16.53	47.70	—	—
24	10.03	671.22	25.30	35.33	—	—
∞	—	723.90	27.29	27.29	—	—

Table II. Calculation of Absorption Parameters from the Plasma Concentrations of Form II of Amobarbital

Time (hr)	C_T (μg/ml)	$[AUC]_{t=0}^{t=T}$ (μg·hr/ml)	$K_E[AUC]_{t=0}^{t=T}$ (μg/ml)	A_T/Vd (μg/ml)	$\frac{(A_T/Vd)_T}{(A_T/Vd)_{max}}$	Percent unabsorbed (%)
0.5	32.89	11.66	0.44	33.33	0.54	46
1	42.23	30.83	1.17	43.40	0.70	30
2	47.36	72.87	2.78	50.14	0.81	19
3	48.72	132.70	5.06	53.78	0.87	13
4	51.73	153.78	5.86	57.59	0.93	7
5	50.69	219.17	8.35	59.04	0.95	5
6	50.24	267.49	10.19	60.43	0.98	2
9	46.51	405.83	15.46	61.97	—	—
12	39.87	530.61	20.22	60.09	—	—
24	20.65	850.67	32.41	53.06	—	—
∞	—	979.51	37.32	37.32	—	—

$$\frac{A_T}{Vd} = C_T + K_E \int_{t=0}^{t=T} C_b \, dt$$
$$= C_T + K_E \cdot (\mathrm{AUC})_{t=0}^{t=T}$$

where A_T is the total quantity of the drug absorbed at time T and C_T is the blood level of the drug at time T.

By plotting the values of A_T/Vd against time T, a maximum value corresponding to A_∞/Vd, *i.e.*, $(A_T/Vd)_{max}$ can be calculated (Figs. 4 and 5). Further, division of individual values of A_T/Vd by $(A_T/Vd)_{max}$ would give the absorption rate at each time. The values obtained by these calculations are given in Tables I and II. Plotting the amount unabsorbed against time gives a first-order absorption curve (Fig. 6), and the absorption rate can be determined from the straight line. As judged from the absorptivity in the initial period in this graph, Form II shows very rapid absorption, about 70% of the drug being absorbed during the first hour, and is much more rapidly absorbed than Form I. It is considered that such a difference in absorptivity in the initial period after administration results in the difference in the subsequent blood levels of Forms I and II. This finding is consistent with the dissolution rates determined *in vitro*, indicating that the different polymorphic crystal forms of amobarbital are absorbed differently through the digestive tract. Thus, it appears that the availability of amobarbital can be increased by utilizing crystal Form II.

References and Notes

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