EFFECTS OF PHENOBARBITAL ON PLASMA CONCENTRATION OF PHENYTOIN AND MORTALITY OF MICE TREATED WITH PHENYTOIN AFTER ELECTROSHOCK

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The effects of phenobarbital on the plasma concentration of phenytoin and mortality of the phenytoin-treated mice were mainly studied. The plasma concentration of phenytoin estimated in the live mice after electroshock was significantly higher than that in the dead mice, suggesting that biological activity of phenytoin is dependent on its plasma concentration where individual difference seems to be large. Though the pretreatment of mice with phenobarbital lowered the plasma concentration of phenytoin, all the mice were alive after electroshock with further treatment with phenobarbital and phenytoin. These results suggest that combination of phenobarbital with phenytoin for the treatment of epileptic patients may be feasible.

Keywords—phenytoin; plasma concentration; phenobarbital; mortality; electroshock

Phenytoin is one of the most frequently used anticonvulsants. There is, however, only a narrow range between the optimum therapeutic and minimally toxic blood concentrations of phenytoin. With the advent of chemical assay techniques, a therapeutic blood concentration of phenytoin and an optimal dose necessary to achieve this concentration can be determined.^{1–3)} Phenytoin and phenobarbital are frequently used in combination in the treatment of epilepsy. Furthermore, a relationship between therapeutic and toxic effects and blood concentrations of phenytoin and of phenobarbital was studied in epileptic patients taking both drugs.⁴⁾ We tested effects of phenobarbital on the plasma concentration of phenytoin and mortality of mice treated with phenytoin to electroshock. In the present study mortality was used as an index of a biological activity of phenytoin because of one of the clear

effects.

Male mice of ddY-strain were fed with solid feed (MM-1, Funabashi Nozuyo) and city service water given freely in a room air-conditioned at 23°C and at a humidity of 65%. The mice, weighing 20 to 30 g were subcutaneously treated with phenytoin (10 mg/kg). At 2.5 h after treatment with phenytoin, the mice were injected with heparin (5 unit/10 g) into *v. coccygenea*. Electroshock was applied to the heparinized mice after 30 min. Electrical stimulation was carried out with a current of 130 V for 0.2 s through the electroshock seizure apparatus devised by Woodbury and Davenport.⁵⁾ All the mice untreated with phenytoin died by 3 h after the shock. After mortality was estimated, 0.5 to 1.0 ml of blood were obtained with a syringe from the heart of the both live and dead mice: immediately after death of mice was confirmed and in live mice at 3 h

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after the electrical shock. Plasma sample was prepared by centrifugation of the blood sample at 3000 rpm for 10 min. Measurement of phenytoin concentration was carried out by the improved method of Saitoh *et al.*⁶⁾ Two millilitres of 1 M phosphate buffer solution (pH 6.8) was added to 0.2 ml of plasma in a centrifuge tube, followed by 10 ml of ethylene dichloride. The mixture was shaken for 10 min and centrifuged at 3000 rpm for 5 min. After the supernatant (aqueous layer) was aspirated off, 9 ml of ethylene dichloride layer was transferred into another centrifuge tube containing 2.5 ml of 0.45 N NaOH. This mixture was shaken for 10 min and again centrifuged. Two millilitres of the NaOH layer was then transferred into a third centrifuge tube. After the addition of 10 ml of 7 N NaOH-4% KMnO₄, the tube was immersed in a water bath of 80°C for 15 min. After cooling, 2 ml of *n*-heptane was added to the solution and the mixture was shaken for 10 min and centrifuged for 5 min. The *n*-heptane layer was transferred into a microcell and the reaction product, benzophenone, was determined spectrophotometrically at 247 nm in *n*-heptane.

To compare the plasma concentrations of phenytoin in the live mice with those in the dead mice, experiments were carried out in 4 groups of 9 to 10 mice. The experimental results (Table I) indicated that a variance between the plasma concentrations of phenytoin obtained from different groups of mice was larger than that within those from the same group of mice. In order to test statistically a difference between the plasma concentrations of phenytoin in the live mice and those in the dead mice, the plasma concentration of it in the each dead mice was expressed as a percentage against its average concentration in the live mice of the same group. The average plasma concentration of phenytoin in the dead mice was $73.5 \pm 10.0\%$ (mean $\pm$ S.E.M.) of that in the live mice (Table I). The plasma concentration of phenytoin estimated in the live mice was significantly higher than that in the dead mice, suggesting that the biological activity of phenytoin is dependent on its plasma concentration, though individual difference seems to be large.

TABLE I. *The Plasma Concentrations of Phenytoin (μ g/ml)*

	Live	Dead
A-group	$4.94 \pm 0.21(8)$	$3.93 \pm 0.92(2)$
B-group	$7.17 \pm 0.63(6)$	$5.50 \pm 1.10(4)$
C-group	$5.53 \pm 0.89(8)$	$1.12(1)$
D-group	$5.18 \pm 0.34(7)$	$4.50 \pm 0.75(2)$
Percent concentration in total mice	100%[29]	$73.5^a \pm 10.0\%$ [9]

Each value is presented as mean $\pm$ S.E.M. (): no. of animals. Percent concentration: see text. []: total of the animals used (a total of animals in A- to D-groups).

a) Significantly different from 100% at $p < 0.05$.

To study the effects of phenobarbital on the plasma concentration of phenytoin and mortality of the phenytoin-treated mice to electroshock, mice were treated with phenobarbital (20 mg/kg, *s. c.*) once a day for 2 (24 and 48 h before the experiments). These mice were termed the phenobarbital-treated mice in this paper. In the following experiments, electrical stimulation was carried out with a current of 80 V for 0.2 sec. Fifty or sixty mice were used for estimation of mortality at 3 h after electroshock. When the mice untreated or treated with phenobarbital were treated with phenytoin, the mortality was 15.0% for the untreated mice and 14.0% for the treated mice, respectively. When the phenobarbital-treated mice, however, were treated with both phenobarbital and phenytoin, all the mice used were alive (Table II).

In the separate experiments the plasma concentration of phenytoin was measured using the same batch of mice. Some investigators⁷⁻⁹⁾ reported that phenobarbital lowered the plasma concentration of phenytoin in clinical study. In contrast to these reports are the findings of Diamond and Buchanan¹⁰⁾ who could not observe any significant stimulation of phenytoin metabolism by phenobarbital. In this study the plasma

TABLE II. *Effects of Phenobarbital on the Plasma Concentration of Phenytoin and Mortality after Electroshock*

Pretreatment with phenobarbital	Drug(s) administered	Mortality percent [no. of dead mice /no. of mice used]	Plasma concentration of phenytoin in live mice ^{a)} [no. of live mice/no. of mice used]
b)	None (Control)	51.7 [31/60]	—
c)	Phenytoin (10 mg/kg) & Phenobarbital (20 mg/kg)	0.0 [0/60]	2.70±0.21 ^{d)} [10/10]
c)	Phenytoin (10 mg/kg)	14.0 [7/50]	2.45±0.31 ^{d)} [6/50]
b)	Phenobarbital (20 mg/kg)	16.6 [10/60]	—
b)	Phenytoin (10 mg/kg)	15.0 [9/60]	3.64±0.26 [9/50]
c)	None	50.0 [25/50]	—

a) Concentration ($\mu\text{g/ml}$) in live mice. b) experiment with mice untreated with phenobarbital. c) Experiment with mice treated with phenobarbital (20 mg/kg) for 2 days. d) Significantly different from the value (3.64 ± 0.62) in the phenobarbital-treated mice at $p < 0.05$.

concentration of phenytoin of the phenobarbital-treated mice after the treatments listed in Table II were significantly lower than that obtained in the mice treated with phenytoin alone (Table II). The plasma concentrations of phenytoin in Table II were obtained in the live mice after electroshock. Decrease in the plasma concentration of phenytoin in the phenobarbital-treated mice (Table II) may be concerned with acceleration in metabolism of phenytoin by stimulation of the liver microsomal enzyme system. Notwithstanding that the plasma concentration of phenytoin was lowered by the pretreatment with phenobarbital, the mortality was 0% for the mice applied phenobarbital and phenytoin in combination (Table II). It can be concluded that the biological effect of phenytoin is potentiated by phenobarbital in this study. However, recent findings of Honda *et al.*⁴⁾ suggested that both therapeutic and toxic effects of phenytoin and phenobarbital were synergistic, when both drugs were used in combination in the treatment of epilepsy. Therefore, dosage schedules should be carefully determined by measurement of the plasma concentrations of two drugs.

Although the plasma concentration of phenytoin in the phenobarbital-treated mice was lower than that in the untreated mice, no change in the effect of phenytoin was observed by the treat-

ment of mice with phenobarbital. This effect of phenytoin in the phenobarbital-treated mice may be due to potentiation by phenobarbital which remained in the body, as the biological half life of phenobarbital is found to be long.¹¹⁾

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