BIOPHARMACEUTICAL STUDIES ON HYDANTOIN DERIVATIVES.II.¹⁾ PHARMACOKINETICS AND BIOAVAILABILITY OF HYDANTOIN DERIVATIVES IN DOGS

HIROSHI FUJIOKA AND TAKASHI TAN

Pharmaceutical Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33-94, 564 Suita, Osaka

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Pharmacokinetics and bioavailability for the 1-benzenesulfonylhydantoin derivatives and 1-unsubstituted hydantoin derivatives were evaluated from the plasma concentrations after oral and intravenous administrations to dogs.

Apparent volume of distribution of the 1-benzenesulfonylhydantoin derivatives was about 0.25 l/kg, while that of the 1-unsubstituted hydantoin derivatives was about 1.3 l/kg. The result suggests that introduction of the benzenesulfonyl group at 1-position of the hydantoin ring has marked effect on the distribution into the fluids and tissues of the body.

Bioavailabilities of sodium 5,5-diphenylhydantoin and sodium 1-benzenesulfonyl-5,5-diphenylhydantoin exceeded those of their corresponding free forms. The results were well explicable in terms of the excellent dissolution behaviors of the salt forms. The bioavailabilities of 5-ethyl-5-phenylhydantoin and 1-benzenesulfonyl-5-ethyl-5-phenylhydantoin were almost perfect. The results suggest that the dissolution rate is a rate-determining step in the bioavailability of the derivatives having two phenyl groups at 5-position of the hydantoin ring, irrespective of the presence of the benzenesulfonyl group at 1-position.

Keywords—hydantoin derivatives; oral and intravenous administrations; plasma concentration; pharmacokinetics; volume of distribution; rate constant of elimination; bioavailability; dog; dissolution behavior; solute-solution equilibrium

In the previous paper,¹⁾ on the basis of the physico-chemical properties of a series of hydantoin derivatives and *in situ* intestinal absorption from aqueous solutions in rats, it was presumed that there would be differences in *in vivo* behaviors between 1-benzenesulfonylhydantoin derivatives and 1-unsubstituted hydantoin derivatives, and that the dissolution rate would be a rate-determining step in the bioavailability for the derivatives having two phenyl groups at 5-position of the hydantoin ring, irrespective of the presence of the benzenesulfonyl group at 1-position.

In order to present unambiguous evidences supporting the presumption, the pharmacokinetics and the bioavailabilities of 5,5-diphenylhydantoin, its sodium salt, 1-benzenesulfonyl-5,5-diphenylhydantoin, its sodium salt, 5-ethyl-5-phenylhydantoin, and 1-benzenesulfonyl-5-ethyl-5-phenylhydantoin were assessed by using

dogs.

It is the purpose of this report to reveal the effects of the benzenesulfonyl group at 1-position and diphenyl groups at 5-position of the hydantoin ring on the pharmacokinetics and bioavailability. Bioavailabilities were discussed in connection with the *in vitro* dissolution behaviors.

So far as 5,5-diphenylhydantoin and its sodium salt are concerned, some reports are available to predict the bioavailability,²⁾ but since the effects of the dosage forms and the particle sizes of the materials are not discussed in these papers, it is not quite clear which form surpasses the other in regard to bioavailability. Hence, the superiority of the salt form over the free form in the bioavailability was newly demonstrated in dogs.

EXPERIMENTAL

Materials — 5,5-Diphenylhydantoin (I), sodi-

um 5,5-diphenylhydantoin (I-Na), 1-benzenesulfonyl-5,5-diphenylhydantoin (II), sodium 1-benzenesulfonyl-5,5-diphenylhydantoin trihydrate (II-Na), 5-ethyl-5-phenylhydantoin (III), and 1-benzenesulfonyl-5-ethyl-5-phenylhydantoin (IV) were synthesized in our research laboratory. Each material was well grounded in agate mortar with a pestle to powder measuring 1–3 micron in diameter, prior to experiments.

Measurement of in Vitro Dissolution Rate — I or I-Na equivalent to 100 mg of I, which would produce the concentration equal to 25 times the solubility of I at pH 7.0 assuming that it is completely dissolved, was added to 100 ml of the isotonic sodium phosphate buffer of pH 7.0 in a 200 ml conical flask and the flask was shaken at the rate of 90 times per minute at the temperature of 37°C. The concentrations of I were determined spectrophotometrically as time lapses, with Hitachi EPS-2U Spectrophotometer. The dissolution rates of II and II-Na were also determined in a similar manner as above.

Experimentals in Dogs — Cross-over tests were carried out on male beagle dogs weighing about 15 kg at 2-week intervals.

Oral Administration: Each material was loaded into a glass tube connected to the upper end of a cannula and was poured with 60 ml of water into the stomach of dogs fasted overnight prior to the experiments. Doses used were as follows: for I and

I-Na, equivalent in amount to 200 mg of I; for II and II-Na, equivalent in amount to 450 mg of II; for III, 300 mg; and for IV, 100 mg.

Intravenous Administration: Solution of each material was injected into the brachial vein. Doses used were as follows: for I-Na, equivalent in amount to 100 mg and 200 mg of I in 10 ml of water; for II-Na, equivalent in amount to 100 mg of II in 10 ml of an aqueous solution of propylene glycol/ethanol/water (4:1:5, v/v); for III-Na, equivalent in amount to 150 mg of III in 10 ml of water; for IV-Na, equivalent in amount to 50 mg of IV in 10 ml of water.

In each experiment, no food was given for 8 h after drug administration and heparinized blood samples were taken at preselected time intervals up to 24 h. Plasma was separated within 30 min by centrifugation.

Analytical Method — Plasma concentrations of II were determined by gas chromatography.¹⁾ Concentrations of I, III, and IV in plasma were also determined by the method used for II with the following modifications in the extraction procedure: (1) isobutyl alcohol-chloroform (1:4) was used in place of chloroform as an organic solvent; (2) 1 ml of plasma was acidified with 1 ml of 1 N HCl and extracted with 10 ml of the organic solvent. Eight milliliters of the organic phase was back-extracted into 10 ml of 0.1 N NaOH. Eight milliliters of the aqueous phase were treated

TABLE I. *Pharmacokinetic Data of Hydantoin Derivatives in Dog*

Compound No.	pK_a	% unionized at pH 7.4	dose mg/dog	(n) ^{a)}	Pharmacokinetic constant ^{b)}		
					Volume of distribution l/kg	First order rate constant of elimination, h ⁻¹	Biological half-life h
I	8.30	88.8	100	6	1.29 ± 0.04	0.191 ± 0.017	3.78 ± 0.32
			200	6	1.23 ± 0.06	0.183 ± 0.013	3.87 ± 0.25
II	4.89	0.308	100	6	0.251 ± 0.006	0.156 ± 0.007	4.51 ± 0.21
III	8.51	92.8	150	2	1.30 ± 0.05	0.031 ± 0.002	22.6 ± 1.5
IV	5.20	0.627	50	2	0.263 ± 0.004	0.316 ± 0.040	2.24 ± 0.31

a) Number of animals used.

b) Values are mean ± SE.

CHART 1. Pharmacokinetic Model for Evaluation of Bioavailability

according to the identical procedure used for II.

RESULTS AND DISCUSSION

Pharmacokinetics of Hydantoin Derivatives

The plasma disappearances of I, II, III, and IV after intravenous administration were well described by the one compartment open model with the first order rate kinetics. The pharmacokinetic parameters estimated from the plasma data are summarized in Table I.

The apparent volume of distribution of about 0.25 l/kg for II and IV suggests that the 1-benzenesulfonylhydantoin derivatives may be mainly confined within the extracellular space, while that of about 1.3 l/kg for I and III is nearly equal to twice the amount of the total body fluids. The difference in the apparent volume of distribution between the 1-benzenesulfonylhydantoin derivatives and the 1-unsubstituted hydantoin derivatives is probably due to their tendency to ionize: the former having pK_a value of about 5 appears almost in the ionized form in the plasma, whereas the latter having pK_a value of about 8.5 exists mainly in the unionized form. These results suggest that the hydantoin derivatives ionized in the plasma are not appreciably distributed into the tissues.

The introduction of the benzenesulfonyl group at 1-position of the hydantoin ring generally does not seem to affect the elimination kinetics. The first order rate constant of elimination of about 0.03 h^{-1} for III is one fifth to one tenth of that for I, II, and IV, suggesting that III is slowly metabolized.

The rate and the extent of bioavailability of I, II, III, and IV after oral administration may be estimated from the plasma concentrations by Eqs. 1 and 2,³⁾ on the basis of the assumption that the

one compartment open model is appropriate for depicting the pharmacokinetics, as shown in Chart 1:

$$\frac{dA}{dt} = \frac{dC}{dt} \cdot V_d + K_{el} \cdot V_d \cdot C \quad \text{Eq. 1}$$

$$A_T = V_d \cdot C_T + K_{el} \cdot V_d \cdot \int_0^T C \cdot dt \quad \text{Eq. 2}$$

where A_T is the cumulative amount absorbed between time zero and some time T ; C is the plasma concentration; V_d is the apparent volume of distribution; K_{el} is the first order rate constant of elimination.

Dose dependency of the plasma half-life of I has been reported in dog⁴⁾ and man,⁵⁾ and the bioavailability in man has been estimated by the nonlinear method with Michaelis-Menten elimination kinetics,⁶⁾ but our results are consistent with the view put forward by H.H. Frey *et al.*⁷⁾ that the decrease in plasma I concentration after intravenous injection was monoexponential and

FIG. 1. Plasma Concentration-Time Curves of I after Oral Administration of I (—○—) or I-Na (—●—), Equivalent to 200 mg of I. Each point is the mean $\pm$ SE for 5 animals.

fitted an open 1-compartment model.

Bioavailability of I and I-Na

Fig.1 shows that the peak plasma concentration of I was substantially higher and more rapidly attained after oral administration of I-Na as

compared to that observed after oral administration of I. The differences in plasma concentrations are statistically significant ($p < 0.01$) up to 2 h after administration.

The rates and extents of bioavailability are

FIG. 2. Mean Rates (Dotted Lines) and Extents (Full Lines) of Bioavailability versus Time for I (Thin Lines) and I-Na (Thick Lines)
Vertical bracketed lines at 8 h represent standard error of the mean.

FIG. 3. Dissolution Profiles for I (—○—) and I-Na (—●—) in Isotonic Phosphate Buffer of pH 7.0

FIG. 4. Plasma Concentration-Time Curves of II after Oral Administration of II (—○—) or II-Na (—●—), Equivalent to 450 mg of II
Each point is the mean $\pm$ SE for 6 animals.

FIG. 5. Mean rates (Dotted Lines) and Extents (Full Lines) of Bioavailability versus Time for II (Thin Lines) and II-Na (Thick Lines)
Vertical bracketed lines at 8 h represent standard error of the mean.

shown in Fig. 2. The rate of bioavailability produced by I-Na was greater than that produced by I, reaching the peak of about 70 mg/h within 30 min. The absorption process lasts 4–5 h in each case. The extents of bioavailability of I-Na and I were about 50% and 30% of the dose, respectively.

The dissolution behaviors of I and I-Na are shown in Fig. 3. The dissolution rate of I-Na was substantially greater than that of I. When the concentration became equal to the solubility of I, 38

μg/ml, a solute-solution equilibrium was established and the precipitate became I, even in the case of I-Na.

FIG. 6. Dissolution Profiles for II (—○—) and II-Na (—●—) in Isotonic Phosphate Buffer of pH 7.0

FIG. 7. Plasma Concentration-Time Curve of III after Oral Administration of 300 mg of III
Each point is the mean $\pm$ SE for 2 animals.

FIG. 8. Mean Rate (---) and Extent (—) of Bioavailability versus Time for III
The vertical bracketed line at 8 h represents the standard error of the mean.

FIG. 9. Plasma Concentration-Time Curve of IV after Oral Administration of 100 mg of IV
Each point is the mean $\pm$ SE for 2 animals.

The observed difference in the dissolution behavior between I and I-Na well reflects on the difference in bioavailability between them.

Bioavailability of II and II-Na

It is clear from Fig. 4 that the plasma concentrations of II following oral administration of II-Na are significantly higher than those produced by II over entire period of sampling.

The rates and extents of bioavailability are shown in Fig. 5. A more rapid rise in the rate of bioavailability was achieved with II-Na than with II, *i.e.* the rate of bioavailability reaching the peak of about 130 mg/h within 1 h with the former and about 50 mg/h at 2 h with the latter. In each case the absorption process lasted 6–7 h. The extents of bioavailability of II-Na and II were about 95% and 45% of the dose, respectively.

As can be seen in Fig. 6, the rate of dissolution of II-Na is much faster than that of II. Although II-Na produced a very stable supersaturated state, the solid-solution equilibrium was finally established when the solid phase was completely converted to II. Since II and II-Na gave finally the same equilibrium solubility, 240 $\mu\text{g/ml}$, the difference in the dissolution behavior between them must be attributed solely to the difference in the dissolution processes leading to the equilibrium state.

The theoretical consideration of the dissolution processes in reactive media has been discussed by W.I. Higuchi *et al.*⁸⁾

A large dissolution rate of II-Na led to its excellent bioavailability.

Bioavailability of III

Fig. 7 shows the plasma concentration-time curve of III following oral administration of III. The rate and extent of bioavailability of III are shown in Fig. 8.

These data suggest that III is rapidly and almost completely absorbed within 2 h and that the dissolution is no longer the rate-determining step in the bioavailability of III.

Bioavailability of IV

Fig. 9 shows the plasma concentration-time curve of IV following oral administration of IV.

The rate and extent of bioavailability of IV shown in Fig. 10 indicate that absorption of IV lasts for 5–6 h and is almost complete.

The main conclusion to be drawn from the results in this study are as follows: (1) As suggested from the physico-chemical properties, the dissolution rate is a rate-determining factor in the bioavailability for the derivatives having two phenyl groups at 5-position of the hydantoin ring, irrespective of the presence of the benzenesulfonyl group at 1-position while the absorption of the 1-benzenesulfonylhydantoin derivatives (II) and (IV) lasts for a longer time than that of the corresponding 1-unsubstituted derivatives (I) and (III); (2) The significant difference in the apparent volume of distribution between the 1-benzenesulfonylhydantoin derivatives and 1-unsubstituted hydantoin derivatives suggests that the former is distributed to a lesser extent in the tissues compared with the latter. This will be established subsequently.

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FIG. 10. Mean Rate (---) and Extent (—) of Bioavailability versus Time for IV

The vertical bracketed line at 8 h represents the standard error of the mean.

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