BIOPHARMACEUTICAL STUDIES ON HYDANTOIN DERIVATIVES. V.¹⁾ PHARMACOKINETICS AND PHARMACODYNAMICS OF 5,5-DIPHENYLHYDANTOIN AND 1-BENZENESULFONYL-5,5-DIPHENYLHYDANTOIN

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Disposition of 1-benzenesulfonyl-5,5-diphenylhydantoin (II) having a potent anti-inflammatory activity was compared with that of 5,5-diphenylhydantoin (I), an antiepileptic drug, in order to elucidate whether the pharmacodynamic difference between them can be explained by their physicochemical and pharmacokinetic properties.

After oral administration of I-¹⁴C to rats, radioactivity was distributed in all tissues including the brain, whereas after II-¹⁴C administration, the concentrations of radioactivity in most tissues were lower than those in plasma. The results were consistent with the finding obtained by whole-body autoradiography which revealed that after oral administration of II-¹⁴C to rats, radioactivity was not transferred into brain but was significantly transferred into inflamed tissues.

Brain/plasma concentration ratio of I was about 1.3, whereas that of II was about 0.05. Plasma protein binding of I having pK_a value of 8.30 was about 88%, whereas that of II having pK_a value of 4.89 was about 99%. The changes in physicochemical properties due to introduction of a benzenesulfonyl group into the hydantoin ring may be responsible for the difference in the disposition between I and II.

When II was cerebroventricularly administered to mice, it showed a potent anti-convulsant activity against maximal electroshock seizure, the activity being comparable to that for I. This indicates that the earlier failure to demonstrate the activity of II in a routine screening test for antiepileptic drugs was due to the inability of II to penetrate the blood-brain barrier and to achieve effective concentration in the brain.

II was found to inhibit the biosynthesis of prostaglandin. These findings along with the physicochemical properties suggest that although II does not fall structurally under any category of anti-inflammatory drugs the mechanism of action may be similar to that for non-steroidal acidic anti-inflammatory drugs.

Keywords — 5,5-diphenylhydantoin; 1-benzenesulfonyl-5,5-diphenylhydantoin; radioisotope; tissue distribution; whole-body autoradiography; physicochemical property; pharmacokinetics; plasma protein binding; anticonvulsant activity; prostaglandin biosynthesis inhibition

INTRODUCTION

5,5-Diphenylhydantoin (I), a widely used antiepileptic drug, has anticonvulsant activities against chemical and electroshock-induced seizures in laboratory animals,²⁾ whereas 1-benzenesulfonyl-5,5-diphenylhydantoin (II), when orally administered, showed no anticonvulsant activity in a routine screening test for antiepileptic drugs. However, II was incidentally found to have potent anti-inflammatory effects in several models of inflammation in laboratory animals.³⁾

From the standpoint of structure-activity relationships, it would be particularly interesting

to investigate why I and II showed such different pharmacodynamic properties. Previous studies revealed that the introduction of a benzenesulfonyl group at 1-N position of the hydantoin ring causes marked changes in the physicochemical properties,⁴⁾ and that bioavailability of I and II is determined by the rate of dissolution in the gastrointestinal tract.⁵⁾

The aim of the present study was to determine if differences in pharmacodynamic profiles between I and II are related to their intrinsic activity or to their pharmacokinetic property, and to clarify the mechanism of action of II. To this

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end, the effects of introduction of a benzenesulfonyl group at 1-N position of the hydantoin ring on the plasma protein binding, on brain and liver distribution and on pharmacokinetic parameters in rats were investigated with the aid of 5-ethyl-5-phenylhydantoin (III) and 1-benzenesulfonyl-5-ethyl-5-phenylhydantoin (IV) as well as I and II. Anticonvulsant activity of II against maximal electroshock seizure following oral, intravenous or intracerebroventricular administration in mice were compared with that of I, and inhibition of prostaglandin biosynthesis by II, with that by conventional anti-inflammatory drugs.

EXPERIMENTAL

Apparatus — Radioactivity was measured with a TEN liquid scintillation counter, model GSL-162 (Fujitsu Co., Ltd.). Quenching was corrected by the channel ratio method. Infrared spectra were taken with a 215 Hitachi Grating Infrared Spectrophotometer. A JEOL JGC-750 Gas Chromatograph equipped with a flame ionization detector was used for the quantitative analysis. A 0.75 m, 3 mm i.d. stainless steel tubing packed with Shimalite 80/100 mesh coated with 3% OV-17 was used as the column. Thin-layer chromatography (TLC) was performed on plates coated with Silica gel G (Merck), 0.2 mm in thickness, with following solvent systems: 1, benzene-acetone (9:1); 2, ethyl ether-ethyl acetate-acetone (1:1:1) and 3, *n*-butanol-acetic acid-water (4:1:1).

Materials — Phenylbutazone, indomethacin and acetylsalicylic acid were purchased from Nakarai Chemicals Ltd., Japan. Arachidonic acid-1-¹⁴C was purchased from Amersham International Ltd., Amersham, England (specific activity 54.6 mCi/mmol). 5,5-Diphenylhydantoin (I), 1-benzenesulfonyl-5,5-diphenylhydantoin (II), 5-ethyl-5-phenylhydantoin (III), 1-benzenesulfonyl-5-ethyl-5-phenylhydantoin (IV) and 1,3-dibenzesulfonyl-5,5-diphenylhydantoin (V) were synthesized in our research laboratories. 5,5-Diphenylhydantoin-4-¹⁴C (I-¹⁴C) was purchased from New England Nuclear Corp., Boston, Mass. (specific activity 4.73 mCi/mmol). 1-Benzenesulfonyl-5,5-diphenylhydantoin-4-¹⁴C (II-¹⁴C) was synthesized, according to the procedure of Umemoto *et al.*⁶⁾ as follows: 1,3-Dibenzesulfonyl-5,5-diphenylhydantoin-4-¹⁴C (V-¹⁴C). A mixture of I-¹⁴C

(0.398 mCi, 21.2 mg, 0.16 mmol), I (19.2 mg, 0.16 mmol), triethylamine (0.21 ml, 1.50 mmol) and benzenesulfonyl chloride (0.09 ml, 0.75 mmol) was heated in acetone (1 ml) in a sealed tube for 4 h at 40 °C, and the tube was kept standing overnight at room temperature. The resulting precipitate was obtained by filtration, washed successively with 1.5 ml of mixture of methanol-water (1:1), 5 ml of water and 1 ml of ethyl ether, and dried *in vacuo*, yielding a white powder of V-¹⁴C (64 mg, 75%). TLC showed only one radioactive spot which corresponded to V. The *R_f* value of V was 0.80 in solvent system 1.

II-¹⁴C — A mixture of V-¹⁴C (64 mg, 0.12 mmol), diethylamine (0.29 ml, 2.88 mmol) and acetone (0.35 ml) was refluxed for 2.4 h. The resulting precipitate was collected by filtration, washed with 2 ml of acetone, and dissolved in 10 ml of hot water. The solution was acidified with dilute hydrochloric acid to produce a precipitate. The filtered precipitate was washed with water, recrystallized from mixture of ethanol-water (5:4), and dried *in vacuo*, 4 mmHg, over P₂O₅ to give white needles of II-¹⁴C (35.0 mg, 77.4%). TLC showed only one radioactive spot which corresponded to that for II. The *R_f* value of II was 0.35 in the solvent system 1 and 0.80 in the solvent system 2. II-¹⁴C was also identified by infrared spectra. Isotope dilution analysis gave a satisfactory result in radiochemical purity. The specific activity was 2.40 mCi/mmol and the radiochemical yield from I-¹⁴C was 55.8%.

All other chemicals (Wako Pure Chemical Co., Japan) were of analytical grade.

Tissue Distribution Studies of ¹⁴C in Rats — Male Wistar rats weighing about 200 g were each given a single oral dose of 100 mg (21 μCi) of I-¹⁴C or II-¹⁴C per kg as a suspension in 5% sodium carboxymethylcellulose (CMC-Na). Each rat had free access to food and water, and was housed singly in a metabolism cage, allowing separate collection of urine and feces. Plasma and tissue collections were made at 2, 6, 24 and 48 h after administration. Blood was collected by cardiac puncture with a heparinized syringe and was immediately centrifuged at 3000 rpm for 10 min.

The biliary excretion of radioactivity was studied in rats with biliary fistula (polyethylene tubing) inserted in the common bile duct, after injection of 11.5 mg (6.4 μCi) of II-¹⁴C per kg in

0.5 ml of an aqueous solution into the femoral vein. II-¹⁴C was administered as the sodium salt but the dose was expressed as the free acid.

Preparation of Samples for Radioactivity Measurement — Tissues: Each tissue was excised, rinsed with saline, blotted with paper and weighed. Tissue samples (0.1–0.2 g) were solubilized in 2 ml of Soluene, and 10 ml of toluene scintillation fluid and 0.4 ml of acetic acid were added.

Plasma: Plasma samples (0.1 ml) were processed as described above for tissues.

Gastrointestinal Contents: Contents were separated from parts of the tract by rinsing with 5 ml of water. The washings were evaporated to dryness *in vacuo* with concentrated sulfuric acid. About 50 mg of the residue were used for determination of radioactivity by the oxygen-flask combustion method.⁷⁾

Feces: About 50 mg of dried feces were used for measurement of radioactivity by the oxygen-flask combustion method.

Urine and Bile: To aliquots of urine and bile samples were added 10 ml of Bray's solution.⁸⁾

In the calculation of recovery of radioactivity in tissues, the plasma volume and muscle weight were assumed to be 4.04% and 45.4% of body weight, based on the data of Altman *et al.*⁹⁾ and Koyama,¹⁰⁾ respectively.

Whole-Body Autoradiography — Male Wistar rats weighing about 130 g were given an oral dose of 100 mg (283 μ Ci) of II-¹⁴C per kg as a suspension in 5% CMC-Na. At 5 h after administration, rats were frozen in dry ice-acetone and sectioned. The 50 μ m thick sections were freeze-dried and processed for autoradiography as described previously.^{11,12)} Granuloma in a pouch on the back of a rat was produced by subcutaneous injection of air and croton oil, according to the procedure of Seyle¹³⁾ and the rat was used for the experiment 5 d after applying croton oil.

Plasma Protein Binding of I, II, III and IV — The solutions of each material for intravenous injection were prepared as described previously.⁵⁾ Male Wistar rats weighing 200 g received 0.5 ml of the solutions *via* femoral vein. For the measurement of plasma protein binding, blood was collected 1 h after administration of 0.5–15 mg of I–IV, by cardiac puncture, and was centrifuged at 3000 rpm for 10 min. In each case an aliquot (8 ml) of pooled plasma from 3 rats was placed in an Amicon Ultrafiltration

Cell, Model 12, with Diaflo Ultrafiltration Membrane PM 30 25 mm and filtered. The first 0.5 ml of filtrate was discarded and the subsequent 0.5 ml was used for assay. For each material, the filtrate volume did not cause any change in plasma concentration before and after filtration. The percentage bound was calculated as follows:

$$\% \text{ bound} = \frac{100 \times (\text{plasma concentration} - \text{concentration in filtrate})}{\text{plasma concentration}}$$

Distribution of I, II, III and IV in the Brain and Liver — In the distribution study, doses were as follows: I, 2.57 mg/rat; II, 4.89 mg/rat; III, 2.68 mg/rat and IV, 2.54 mg/rat. At appropriate time intervals after injection into femoral vein, rats were sacrificed by drawing blood *via* cardiac puncture and plasma was separated by centrifugation. The brain and liver were excised, blotted dry, weighed and homogenized. Plasma concentrations of I, II, III and IV were determined by gas chromatography described previously.⁵⁾ Tissue concentrations of I, II, III and IV were also determined by gas chromatography as follows: whole brain (about 1.7 g) and a portion of liver (about 2.5 g) were each homogenized with 4 ml of saline. After transfer of the homogenate to a 40 ml centrifuge tube containing 1 ml of 6 N HCl and 8 ml of isobutyl alcohol-chloroform (1:4) mixture, the tube was mechanically shaken for 20 min and centrifuged. A 6 ml aliquot of the organic solvent phase was transferred to another centrifuge tube containing 8 ml of 1 N NaOH. After the tube was shaken and centrifuged, 6 ml of the aqueous phase was transferred to another tube containing 3 ml of 6 N HCl and 5 ml of isobutyl alcohol-chloroform (1:4) mixture. The tube was shaken and centrifuged. A 4 ml aliquot of the organic solvent phase was evaporated to dryness. The residue was analyzed by gas chromatograph.⁴⁾

Measurement of Anticonvulsant Activity — Male mice of Std: ddy strain weighing 20–24 g in groups of 5–10 each were given various doses of I or II by routes of intracerebroventricular, intravenous or oral administration. Intraventricular administration was done according to the method of Haley and McCormick.¹⁴⁾ At an appropriate time after administration, the animals were subjected to 60 Hz current of 25 mA for 0.2 s delivered through corneal

electrodes. Complete abolition of the tonic hind-limb extensor component of seizures was taken as evidence of anticonvulsant activity.

Prostaglandin Biosynthesis — The renal medulla was isolated from the renal cortex of rabbits (2.5–3.5 kg) and homogenized in 3 volumes of 0.1 mol/l phosphate buffer (pH 7.4). After centrifugation at $12000 \times g$ for 20 min, the supernatant fluids were recentrifuged at $105000 \times g$ for 60 min. The pellets were suspended in the phosphate buffer and the suspensions were again centrifuged at $105000 \times g$ for 60 min. The pellets were resuspended in the phosphate buffer, lyophilized and stored in a freezer at -58°C until use. These lyophilized microsomes were used as the microsomal enzyme preparation.¹⁵⁾ Each incubation mixture (total volume 0.4 ml) contained 0.33 mmol/l arachidonic acid-1- ^{14}C (0.2 μCi), 1.7 mmol/l ethylenediaminetetraacetic acid (EDTA)-2Na, an appropriate concentration of drug, and 2.0 mg of microsomal enzyme preparation in 0.1 mol/l phosphate buffer (pH 7.4). After incubation at 37°C for 30 min, the reaction was terminated by the addition of 0.2 ml of 0.1 N HCl. The samples were extracted with ethyl acetate, dried under a stream of nitrogen, and then redissolved in 50 μl of ethanol. The ethanol solution was spotted on a TLC plate and the plate was developed in a solvent system of ethyl ether-acetone-acetic acid (80:15:0.5). The developed plate was scanned in a radiochromatogram scanner (Packard, Model 7201). The amount of prostaglandin E_2 (PGE_2) synthesized was calculated from the ratio of the peak area corresponding to PGE_2 to the total peak area. The PGE_2 standard was cochromatographed and visualized with iodine vapor. The values for the drugs were obtained by running duplicate or triplicate assays. IC_{50} -values represent the molar concentration of the drugs resulting in a 50% inhibition of PGE_2 biosynthesis.

RESULTS AND DISCUSSION

Distribution and Elimination of Radioactivity in Rats after Oral Administration of I- ^{14}C and II- ^{14}C

Table I shows the concentrations of radioactivity in various tissues after oral administration of I- ^{14}C . Table II summarizes recoveries of radioactivity in main tissues, contents of gastrointestinal tracts, urine and feces after oral dose I- ^{14}C . Concentrations of radioactivity in plasma and

most tissues were maximal at 2 h, decreased slightly at 6 h and diminished at 24 h to values that were about 3% or less of those at 6 h. No rat showed retention of radioactivity in tissues at 48 h. Results indicate that radioactivity was well distributed in all tissues including the brain. High concentrations in the liver and kidney appear to be consistent with the finding¹⁶⁾ that I- ^{14}C was mostly metabolized in liver and the metabolites were finally excreted by the renal route. Table II indicates that most of the oral dose was excreted within 48 h after administration, with 47% in urine and 53% in feces, respectively. Analysis of fecal extracts by TLC revealed that radioactivity in feces was exclusively attributed to unchanged I- ^{14}C . Assuming that I- ^{14}C in feces was the unabsorbed fraction, the absorption of I- ^{14}C can be estimated to be about 50% of the oral dose.

On the other hand, as shown in Table III, the concentrations of radioactivity in plasma and most tissues after oral administration of II- ^{14}C reached the maximum at 6 h and decreased at 24 h to about one half of those at 6 h. Even after 48 h, appreciable amounts of radioactivity remained in the liver and intestinal contents. Concentrations of radioactivity in most tissues were lower than those in plasma. Particularly, the concentrations in the brain were found to be only about 5% of those in plasma. Recoveries of radioactivity after oral administration of II- ^{14}C , as shown in Table IV, indicate that radioactivity was mostly excreted in feces. However, radioactivity in feces was found to be derived from the biliary excretion of the absorbed fraction, based on the results showing that radioactivity in plasma and tissues at 6 h amounted to be about 40% of the dose and that the biliary excretion of radioactivity until 24 h after intravenous injection of II- ^{14}C amounted to 83% of the dose. Furthermore, TLC revealed that the bile contained considerable amounts of metabolites of II- ^{14}C with a trace amount of unchanged II- ^{14}C . These results suggest that metabolism followed by biliary excretion was the major route of elimination of II- ^{14}C . Detail on the metabolism of II will be reported separately.

As shown in Fig. 1, the whole-body autoradiographic findings after oral administration of II- ^{14}C were consistent with the data summarized in Table III. No significant difference was found in the patterns of the distribution of radioactivi-

ty between a normal rat and a rat with inflammatory granuloma caused by croton oil. No radioac-

tivity was found in the brain but significant radioactivity can be seen in the granuloma.

TABLE I. Concentrations of Radioactivity in Tissues and Plasma of Rats after Oral Administration of $I-^{14}C$

	Concentration ^{a)}			
	2 h	6 h	24 h	48 h
Plasma	15.4 ± 5.5	14.0 ± 4.0	0.5 ± 0.2	0
Liver	49.8 ± 28.1	33.8 ± 2.0	2.4 ± 0.4	0
Kidney	33.4 ± 13.5	31.1 ± 14.0	0.8 ± 0.5	0
Adrenal	29.0 ± 15.0	22.3 ± 8.6	0.1 ± 0.1	0
Spleen	11.2 ± 4.9	7.7 ± 1.3	0.4 ± 0.4	0
Heart	18.6 ± 11.0	14.2 ± 5.8	0.1 ± 0.1	0
Lung	11.1 ± 1.0	7.3 ± 6.2	0	0
Cerebrum	7.5 ± 5.2	6.4 ± 3.4	0.1 ± 0.1	0
Cerebellum	7.7 ± 4.4	6.8 ± 3.8	0.1 ± 0.1	0
Fat	12.6 ± 5.9	14.3 ± 1.0	1.7 ± 1.7	0
Muscle	12.1 ± 6.6	9.8 ± 3.4	0.3 ± 0.2	0
Submaxillary gland	17.0 ± 10.6	14.1 ± 5.2	0.2 ± 0.2	0
Testicle	8.1 ± 4.2	7.1 ± 2.3	0.2 ± 0.2	0
Seminal vesicle	11.7 ± 6.3	9.3 ± 3.1	0.2 ± 0.2	0
Stomach	41.3 ± 5.0	38.4 ± 10.1	2.9 ± 2.5	0
Large intestine	13.3 ± 5.6	47.4 ± 5.5	3.2 ± 2.5	0
Small intestine	54.6 ± 18.2	61.1 ± 25.1	2.8 ± 1.5	0
Caecum	34.9 ± 34.9	201 ± 141	9.2 ± 4.5	0

Dose of $I-^{14}C$ was 100 mg/kg. a) Tissue and plasma concentrations are expressed as μg eq of I/g of tissue and as μg eq of I/ml of plasma, respectively. Data are presented as mean values $\pm$ S.D. for three animals.

TABLE II. Recoveries of Radioactivity in Tissues, Contents of Gastrointestinal Tract and Excreta of Rats after Oral Administration of $I-^{14}C$

	Recovery ^{a)}			
	2 h	6 h	24 h	48 h
Plasma	0.6 ± 0.2	0.6 ± 0.2	0	0
Liver	2.3 ± 1.4	1.5 ± 0.1	0.1 ± 0.0	0
Kidney	0.3 ± 0.2	0.3 ± 0.1	0	0
Muscle	5.7 ± 0.0	5.0 ± 0.0	2.4 ± 0.0	0
Lung	0.1 ± 0.1	0	0	0
Small intestine	1.9 ± 0.6	2.5 ± 1.0	0.1 ± 0.0	0
Caecum	0.2 ± 0.2	1.0 ± 0.6	0	0
The rest	0.5 ± 0.0	0.7 ± 0.2	0	0
Contents of stomach	39.2 ± 1.8	13.9 ± 7.1	0.1 ± 0.1	0
Contents of large intestine	1.1 ± 1.1	6.6 ± 2.2	0.2 ± 0.2	0
Contents of small intestine	31.9 ± 5.4	15.6 ± 4.0	0.1 ± 0.1	0
Contents of caecum	8.3 ± 8.3	24.8 ± 12.7	0.4 ± 0.4	0
Urine	5.1 ± 4.5	9.5 ± 6.6	32.9 ± 3.2	47.0 ± 7.8
Feces	0	5.1 ± 4.0	58.5 ± 5.1	53.0 ± 9.7
Total recovery	91.8 ± 7.2	77.8 ± 6.8	95.0 ± 3.0	103.8 ± 6.0

Dose of $I-^{14}C$ was 100 mg/kg. a) Recoveries are given as percentage of dose. Data are presented as mean values $\pm$ S.D. for three animals.

Effects of Physicochemical Properties and Plasma Protein Binding on the Distributions of I, II, III and IV in the Brain and Liver of Rats
Studies on the dispositions of radioactivity

TABLE III. Concentrations of Radioactivity in Tissues and Plasma of Rats after Oral Administration of II-¹⁴C

	Concentration ^{a)}			
	2 h	6 h	24 h	48 h
Plasma	80.3 ± 28.4	210.1 ± 38.5	104.3 ± 23.5	0.6 ± 0.2
Liver	119.6 ± 39.6	194.6 ± 41.2	143.6 ± 20.1	9.8 ± 1.2
Kidney	42.5 ± 5.5	109.9 ± 12.0	79.3 ± 4.4	2.4 ± 1.9
Adrenal	47.8 ± 11.8	115.4 ± 21.3	74.4 ± 18.3	0.2 ± 0.1
Spleen	6.0 ± 4.1	40.5 ± 7.9	20.0 ± 8.0	0.1 ± 0.1
Heart	32.9 ± 14.1	102.9 ± 22.7	65.0 ± 4.6	0.9 ± 0.3
Lung	28.3 ± 10.8	103.8 ± 9.8	50.1 ± 7.1	0.3 ± 0.2
Cerebrum	4.5 ± 1.5	18.7 ± 4.1	9.1 ± 3.6	0.2 ± 0.1
Cerebellum	4.9 ± 2.9	18.1 ± 3.7	7.6 ± 1.3	0.2 ± 0.1
Fat	13.8 ± 6.5	40.5 ± 7.5	24.5 ± 5.2	0.4 ± 0.1
Muscle	14.5 ± 4.1	44.0 ± 11.0	25.0 ± 4.3	0.7 ± 0.1
Submaxillary gland	34.7 ± 10.5	100.0 ± 18.3	56.7 ± 5.8	0.7 ± 0.1
Testicle	7.4 ± 2.9	39.2 ± 9.0	27.9 ± 8.5	0.2 ± 0.2
Seminal vesicle	9.7 ± 3.5	29.9 ± 4.4	22.8 ± 4.4	0.3 ± 0.3
Stomach	59.2 ± 12.4	85.8 ± 4.0	62.3 ± 24.6	0.6 ± 0.3
Large intestine	17.3 ± 4.0	56.8 ± 19.1	80.6 ± 25.5	3.6 ± 1.9
Small intestine	115.3 ± 21.3	108.1 ± 20.2	129.7 ± 39.3	2.7 ± 2.7
Caecum	13.2 ± 8.6	228.0 ± 24.8	393.2 ± 210.8	11.4 ± 7.1

Dose of II-¹⁴C was 100 mg/kg. a) Tissue and plasma concentrations are expressed as $\mu\text{g eq}$ of II/g of tissue and as $\mu\text{g eq}$ of II/ml of plasma, respectively. Data are presented as mean values $\pm$ S.D. for three animals.

TABLE IV. Recoveries of Radioactivity in Tissues, Contents of Gastrointestinal Tract and Excreta of Rats after Oral Administration of II-¹⁴C

	Recovery ^{a)}			
	2 h	6 h	24 h	48 h
Plasma	3.1 ± 1.1	8.0 ± 1.4	3.8 ± 1.2	0
Liver	4.3 ± 1.4	6.1 ± 1.0	4.5 ± 0.7	0.5 ± 0.1
Kidney	0.2 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0
Muscle	6.6 ± 1.9	20.0 ± 5.0	11.2 ± 2.0	0.3 ± 0.1
Lung	0.1 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0
Small intestine	2.8 ± 0.7	2.7 ± 0.6	2.9 ± 1.1	0.1 ± 0.1
Caecum	0.1 ± 0.1	1.1 ± 0.3	1.4 ± 0.8	0
The rest	0.7 ± 0.3	2.0 ± 0.7	1.7 ± 0.8	0
Contents of stomach	47.6 ± 12.4	37.2 ± 21.2	7.0 ± 7.0	0
Contents of large intestine	0	0.2 ± 0.1	2.5 ± 2.5	0.2 ± 0.2
Contents of small intestine	32.4 ± 13.9	8.2 ± 1.0	4.9 ± 0.9	1.3 ± 1.3
Contents of caecum	0.1 ± 0.0	16.2 ± 10.0	11.9 ± 2.0	0.4 ± 0.4
Urine	0	0.3 ± 0.1	4.4 ± 0.9	4.4 ± 0.9
Feces	0	3.3 ± 2.5	58.5 ± 5.1	67.8 ± 4.1
Total recovery	98.2 ± 11.7	105.3 ± 15.2	87.5 ± 13.2	75.0 ± 1.5

Dose of II-¹⁴C was 100 mg/kg. a) Recoveries are given as percentage of dose. Data are presented as mean values $\pm$ S.D. for three animals.

after oral administration of I- ^{14}C and II- ^{14}C suggest that radioactivity from the latter is more localized in plasma and less distributed in tissues than that from the former. Therefore, by selecting two kinds of groups viz., 1-benzenesulfonylhydantoin, II and IV, and hydantoin without the benzenesulfonyl group, I and III, influences of their physicochemical properties on their distribution into the brain and liver of rats were investigated.

Physicochemical properties^{4,5)} and pharmacokinetic parameters for I, II, III and IV are summarized in Table V.

Liver/plasma and brain/plasma concentration ratios for I, II, III and IV are shown in Figs. 2–5, respectively. Disappearances of I and III from tissues and plasma after intravenous administra-

tion were well described by the one-compartment open model with the first order rate kinetics (data not shown) and their tissue/plasma concentration ratios remained unchanged with time. On the other hand, liver/plasma concentration ratios for II and IV gradually increased with time. This may be explained by the restriction of transfer of II and IV from plasma to liver due to high degree of ionization and high protein binding in plasma and by the high binding affinity of II and IV to tissues of liver.

The brain/plasma concentration ratios of 1.3–1.5 for I and III indicate that they can freely penetrate the blood-brain barrier, whereas those for II and IV were extremely low, indicating that their passage across the barrier is restricted. These results indicate that I and III having

FIG. 1. Autoradiograms Showing the Distribution of Radioactivity after Oral Administration of II- ^{14}C in a Normal Rat (a) and a Rat with Granuloma Induced by Croton Oil (b)

TABLE V. *Physicochemical Properties* ^{4,5)} *and Pharmacokinetic Constants in Rats for I, II, III and IV*

		Compounds			
		I	II	III	IV
pK_a		8.30	4.89	8.51	5.20
Partition ratio ^{a)}		33.8	129	2.69	1.23
Partition coefficient ^{b)}		38.0	41900	2.90	195
% unionized at pH 7.4		88.8	0.308	92.8	0.627
V_d ^{c)} l/kg		0.818	0.281	0.979	0.281
Plasma	K_{el} ^{d)} h ⁻¹	0.476	0.388	0.099	0.618
	$T_{1/2}$ ^{e)} h	1.46	1.78	6.97	1.12
	r^2 ($n = 9$)	0.992	0.944	0.989	0.978
Brain	K_{el} h ⁻¹	0.486	0.395	0.117	0.549
	$T_{1/2}$ h	1.42	1.76	5.91	1.26
	r^2 ($n = 9$)	0.986	0.934	0.992	0.980
Liver	K_{el} h ⁻¹	0.486	0.169	0.110	0.325
	$T_{1/2}$ h	1.43	4.10	6.29	2.13
	r^2 ($n = 9$)	0.992	0.773	0.918	0.978

a) Values in chloroform – pH 7.4 buffer system.
 water system.

c) Volume of distribution.

b) Values for unionized species in chloroform –
 water system.

d) First order rate constant of elimination.

e) Half-life of elimination.

f) Correlation coefficient in first order – regression analysis of elimination rate.

anticonvulsant activity penetrate readily into the brain, whereas II and IV, showing no anticonvulsant activity after oral administration, do not.

The volume of distribution for I and III was 0.8–1.0 l/kg, suggesting their distribution into intracellular fluids, whereas that for II and IV was 0.28 l/kg, suggesting that their distribution is confined to the extracellular space.

Plasma protein bindings of I–IV, determined after intravenous administration to rats, are shown in Fig. 6. Percentages of I, II and IV bound to plasma protein were about 89, 99 and 92%, respectively, and remained essentially constant over wide ranges of plasma concentration, whereas that for III decreased considerably with increasing plasma concentration of III in a range of 5–50 $\mu\text{g/ml}$, this being presumably due to

FIG. 2. *Tissue/Plasma Concentration Ratio – Time Profiles for I in Rats after Intravenous Administration of 2.57 mg of I/Rat*

○, liver/plasma concentration ratio; ●, brain/plasma concentration ratio.

FIG. 3. *Tissue/Plasma Concentration Ratio – Time Profiles for II in Rats after Intravenous Administration of 4.89 mg of II/Rat*

○, liver/plasma concentration ratio; ●, brain/plasma concentration ratio.

saturation of binding sites on plasma proteins. III which has the smallest partition coefficient and the lowest binding affinity for plasma protein and exists almost in the unionized form in plasma is particularly interesting for its longest elimination half-life of all the compounds used.

It is assumed that the inability of II and IV to penetrate into the brain is probably due to their high degree of ionization, 99.7% for II and 99.4% for IV, rather than to plasma protein binding. I can freely penetrate into the brain de-

spite its high percentage bound to plasma protein and also the brain/plasma concentration ratio for III, which showed concentration-dependent plasma protein binding, was not dependent on the plasma concentration. However, in the case of II, the participation of the strong binding to plasma protein and the sequestering in lipid matrixes constituting the blood-brain barrier, as would be expected from the extremely large partition coefficient of the unionized molecule between organic solvent and aqueous buffer, should not be ignored.

Intrinsic Activity of II

In order to clarify whether II has no anticonvulsant activity or orally administered II lacks anticonvulsant effects due to its inability to penetrate the blood-brain barrier, the anticonvulsant activities of I and II were compared after intracerebroventricular administration in mice by a maximal electroshock method. The results are summarized in Table VI, along with those obtained after oral or intravenous administration.

In contrast to the results seen with orally or intravenously administered II, intracerebroventricularly administered II produced a marked anticonvulsant activity. The intensity of effect was found to be dose-related in the range of 0.1–0.8 $\mu\text{M}/\text{mouse}$, the dose-effect curve being sigmoid. The potency of II was essentially the same as that for I.

FIG. 4. *Tissue/Plasma Concentration Ratio - Time Profiles for III in Rats after Intravenous Administration of 2.68 mg of III/Rat*

○, liver/plasma concentration ratio; ●, brain/plasma concentration ratio.

FIG. 5. *Tissue/Plasma Concentration Ratio - Time Profiles for IV in Rats after Intravenous Administration of 2.54 mg of IV/Rat*

○, liver/plasma concentration ratio; ●, brain/plasma concentration ratio.

FIG. 6. *Protein Binding of I, II, III and IV in Plasma after Intravenous Administration of I, II, III or IV to Rats*

○, I; ●, II; □, III; ■, IV.

As shown in Table VII, the time-courses of anticonvulsant effect of I and II were nearly equal, although the effect of II occurred slightly slower than that of I and the subsequent plateau peak was noted between 2 and 4 h after dosing of II, while the peak for I was reached at 2 h and declined thereafter. These results indicate that the intrinsic anticonvulsant activity of II is essentially equal to that of I and that the earlier failure to demonstrate the activity in the pharmacological screening test for antiepileptic drugs is due to its physicochemical properties, leading to its inability to penetrate the blood-brain barrier. This also explains the finding¹⁷⁾ that the introduction of electron attractive groups into 1-N position of the hydantoin ring causes the disappearance of the anticonvulsant activity.

II is a weak acid with pK_a value of 4.89. It is

highly ionized in plasma and highly bound to plasma protein. It inhibits the heat-denaturation of serum protein.³⁾ The volume of distribution is about 0.28 l/kg, suggesting that the distribution is restricted to extracellular fluids. These characteristics of II appear to be peculiar to all the acidic non-steroidal anti-inflammatory drugs.^{18,21)}

In addition, II has proved to possess an inhibitory activity against prostaglandin biosynthesis. Table VIII shows the data on the inhibitory potencies of II and other anti-inflammatory drugs against prostaglandin synthetase from rabbit renal medulla microsome. In this system, II showed a potency comparable to that of acetylsalicylic acid at the concentration of 10^{-3} M (solubility limit of II). Since the hypothesis proposed by Vane,¹⁹⁾ that this anti-enzyme action is responsible for the therapeutic effects of non-

TABLE VI. *Dose-Dependency of Anticonvulsant Activity of I and II against Maximal Electroshock in Mice after Intracerebroventricular (i.c.v.), Intravenous (i.v.) or Oral (p.o.) Administration*

Compound	Dose (μ M/mouse)	Route of administration	Time of test (h)	Response	
				Number of protection / Number of animals	
I	0.1	<i>i.c.v.</i>	2	0/10	
	0.2	<i>i.c.v.</i>	2	0/10	
	0.3	<i>i.c.v.</i>	2	2/10	
	0.4	<i>i.c.v.</i>	2	7/10	
	0.8	<i>i.c.v.</i>	2	10/10	
II	0.1	<i>i.c.v.</i>	3	0/10	
	0.2	<i>i.c.v.</i>	3	2/10	
	0.4	<i>i.c.v.</i>	3	6/10	
	0.8	<i>i.c.v.</i>	3	8/10	
	2.5	<i>i.v.</i>	3	0/ 5	
	5.0	<i>i.v.</i>	3	0/ 5	
	100 ^{a)}	<i>p.o.</i>	2 and 4	0/ 5	
	200 ^{a)}	<i>p.o.</i>	2 and 4	0/ 5	
	400 ^{a)}	<i>p.o.</i>	2 and 4	0/ 5	

a) Dose unit, mg/kg.

TABLE VII. *Time-Course of Anticonvulsant Activity of I and II against Maximal Electroshock in Mice after Intracerebroventricular Administration^{a)}*

Compound	Response						
	Number of protection / Number of animals						
	Time after administration (h)						
	0.5	1.0	1.5	2.0	3.0	4.0	6.0
I	0/10	2/10	4/10	6/10	5/10	4/10	1/10
II	0/10	0/10	2/10	4/10	4/10	4/10	1/10

a) Dose, 0.4 μ M/mouse.

TABLE VIII. Inhibitory Activity of II, Phenylbutazone, Acetylsalicylic Acid and Indomethacin on Prostaglandin E_2 Biosynthesis by Rabbit Renal Medulla

Compound	Conc. ^{a)} (M)	N ^{b)}	Percent inhibition (mean $\pm$ S.E.)	IC ₅₀ in μ M (95% confidence limits)
II	10 ⁻⁴	2	11.3 $\pm$ 2.1	>1000
	3 $\times$ 10 ⁻⁴	5	15.4 $\pm$ 3.4	
	10 ⁻³	2	34.5 $\pm$ 2.8	
Phenylbutazone	10 ⁻⁵	4	20.0 $\pm$ 10.8	26.2 (19.4–35.4)
	3 $\times$ 10 ⁻⁵	4	56.5 $\pm$ 3.8	
	10 ⁻⁴	4	88.6 $\pm$ 4.7	
Acetylsalicylic acid	10 ⁻³	4	22.2 $\pm$ 6.6	2410 (1600–3510)
	3 $\times$ 10 ⁻³	4	53.6 $\pm$ 8.7	
	10 ⁻²	4	85.3 $\pm$ 2.3	
	3 $\times$ 10 ⁻²	4	100.0 $\pm$ 0.0	
Indomethacin	3 $\times$ 10 ⁻⁷	2	6.1 $\pm$ 15.0	2.14 (1.82–2.57)
	10 ⁻⁶	8	28.1 $\pm$ 4.3	
	3 $\times$ 10 ⁻⁶	8	63.3 $\pm$ 2.9	
	10 ⁻⁵	8	85.6 $\pm$ 1.8	
	3 $\times$ 10 ⁻⁵	6	97.4 $\pm$ 1.2	

a) Drug concentration.

b) Number of experiments.

steroidal acidic anti-inflammatory drugs, is generally accepted, the results obtained in this study suggest that although II does not fall structurally under any category of conventional anti-inflammatory drugs, most of its peripheral anti-inflammatory effects may be explained by a mechanism similar to that for other conventional acidic non-steroidal anti-inflammatory drugs.

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