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Blood Concentration and Urinary Excretion Profiles following Oral Administration of Esters of Pyrithioxin to Dogs[1,2]

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An attempt was made to determine and compare oral absorption characteristics of ester derivatives of pyrithioxin (I) filled in hard gelatin capsules after administration to dogs. Ester derivatives of I studied were pyrithioxin-tetraacetate dihydrochloride (I–A·2HCl), pyrithioxin-tetrapropionate dihydrochloride (I–P·2HCl), pyrithioxin-tetra-*n*-butyrate dihydrochloride (I–B·2HCl), and pyrithioxin-4,4′-disuccinate dihydrochloride (I–S·2HCl). Blood concentrations of 5′-desoxy-5′-methylsulfinylpyridoxol (II) and urinary excretions of metabolites of I were studied following oral administration of esters. The values of area under the blood concentration-time curve (AUC) and total urinary excretion in terms of mole percent of dose were used as provisional bioavailabilities of the ester and compared with those of I·2HCl. The bioavailability of I–P·2HCl significantly increased with the maximum blood concentration 1.63 times larger and the total urinary excretion 1.47 times larger than those of I·2HCl. There appeared to be a close relationship between the improved bioavailability of I–P·2HCl and its large partition coefficients of *n*-octanol/pH 6.5 buffer. The insufficient bioavailability of I–S·2HCl appeared to be closely correlated with its low partition coefficient of 0.094 and incomplete hydrolysis of ester during absorption. The esters were considered to be hydrolyzed in intestinal fluid and/or in the intestinal membrane and the parent drug, I, permeated through the intestinal tract. The uptake of ^{14}C-labelled glucose into the brain of the mouse was preliminary studied with I–A·2HCl and I–S·2HCl. I–A·2HCl indicated a significant increase of glucose through the blood-brain barrier similar to that of I·2HCl, but not with I–S·2HCl.

Keywords——pyrithioxin; pro-drug; absorption; urinary excretion; solubility; partition coefficient; pharmacological activity

Recently, many workers have come to be interested in the development of pro-drugs to improve inadequate properties of parent drugs.[4] To guarantee a uniform therapeutic effect with an improved bioavailability, chemical modifications to pro-drugs are adopted regardless of pharmacologically inactive compounds. Pro-drugs generally restore their pharmacological activity by the disjunction of disposable moieties with the autogenous enzyme system and/or other suitable conditions found in the living body. Many pro-drugs have been developed to improve a gastrointestinal absorption resulting in good bioavailability and pharmacological efficacy.[5]

Previous studies from this laboratory[6] indicated that pyridoxine mono- and di-acylates were absorbed through the gastrointestinal tract in man following single oral administrations.

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[1] Biopharmaceutics of Pyrithioxin. III. Part II: K. Kitao, N. Yata, K. Kubo, H. Fujiwara, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **25**, 1343 (1977).

[2] A part of this work was presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, Apr. 1976.

[3] Location: *Yamadakami, Suita, Osaka.*

[4] R.E. Notari, *J. Pharm. Sci.*, **62**, 865 (1973); V.J. Stella, *Aust. J. Pharm. Sci.*, **NS 2**, 57 (1973); A.A. Sinkula and S.H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1976); T. Higuchi and V. Stella, "Pro-drugs as Novel Drug Delivery Systems," Amer. Chem. Soc., Washington, D.C., 1975.

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[6] N. Mizuno, M. Aoki, and A. Kamada, *Bitamin*, **38**, 129 (1968).

The absorbability of the derivatives was closely correlated with enzymatic hydrolysis of acylates in the gastrointestinal tract of man.

The objective of this work were to investigate the feasibility of ester derivatives of pyrithioxin (I) to improve an intestinal absorption and bioavailability following a single oral administration to dog and to clarify a possible relationship between the availability and chemical properties of the derivatives.

Experimental

Materials——I was obtained through the courtecy of the Chugai Pharmaceutical Co., Ltd.

Synthesis of Esters——Pyrithioxin-tetraacetate dihydrochloride (I–A·2HCl), pyrithioxin-tetrapropionate dihydrochloride (I–P·2HCl), pyrithioxin-tetra-*n*-butyrate dihydrochloride (I–B·2HCl), and pyrithioxin-4,4′-disuccinate dihydrochloride (I–S·2HCl) were synthesized following known procedures employing I and anhydrides of the respective above acids and precipitated as dihydrochlorides with HCl-ethanol solution and purified by recrystallization from ethanol. The structures of esters were confirmed with an elementary analysis, colorimetric assay of phenol, infrared spectrum, ultraviolet spectrum, and nuclear magnetic resonance.

Oral Administration——Adult beagle dogs weighing 11—12 kg were kept fast for 12 hr prior to the experiments but water was given *ad libitum*. Single oral dose of I·2HCl and esters, filled in hard gelatin capsules (Matsuya Co., Ltd. No. 0), was administered at a dose level equivalent to 50 mg of I/kg with 30 ml of water. Dogs were not fed until 10 hr after drug administration, but water was given *ad libitum*. At least one week was allowed between drug administrations.

Blood and Urine Samples——Blood and urine samples were collected and prepared following the method described previously.[7]

Drug Transfer through Everted Rat Intestine——Permeation of I and esters through the everted rat intestine was studied following a modified method of Crane and Wilson previously described.[1]

Assay of I and Its Metabolites——I and its metabolites, I-glucuronide (I–G), 5′-desoxy-5′-methylsulfinylpyridoxol (II), and its glucuronide (II–G) were assayed following the method described previously employing a high-performance liquid chromatography (HPLC).[7] The calculation of mole percent excretion in the urine was described in the previous report.[7]

pH-Profile of Partition Coefficient——Partition coefficients of I and esters between *n*-octyl alcohol and water were obtained employing phosphate buffer with a pH range from 2 to 8.5 and an ionic strength of 0.15. *n*-Octyl alcohol and buffer were saturated each other before use. Ten ml of buffer solution containing 20 μм of I·2HCl, or I–S·2HCl was taken into an L-shaped test tube with a glass stopper. An equal volume of *n*-octyl alcohol was placed on the water phase. After shaking at 37° for 1 hr, the concentration of the drug in the water phase was spectrophotometrically measured at 296 nm after being acidified with 0.5 N HCl. For the experiments of I–P, 50 ml of phosphate buffer and 5 ml of 1 mм *n*-octyl alcohol solution of I–P·2HCl were shaken.

Transfer of ^{14}C-labelled Glucose through Blood-Brain Barrier——Male mice, ddy strain, weighing 28±2 g were used. Four groups of animals, 6 animals in each group, were given either saline solutions of I·2 HCl, I–A·2HCl, I–S·2HCl, or a saline solution alone. After daily oral administrations of the solutions at a dose of 0.01 ml/g body weight at 9:00 a.m. over a period of 13 days, the animals were kept fast for 19 hr prior to the experiment but water was given *ad libitum*. The last oral administration was given at 9:00 a.m. of the fourteenth day. At 2:00 p.m., each animal was intraperitoneally injected 10 μC of ^{14}C-labelled glucose. The dose was 0.1 ml of aqueous solution and equivalent to 50 mμ moles of glucose. The specific radioactivity of ^{14}C-labelled glucose used was 190 mC/mmole. The animals were frozen to death with liquid nitrogen exactly 15 min after administration of glucose. The cerebra were immediately removed, taken into glass-stoppered weighing bottles, and kept at −20°. Each cerebrum was homogenized in 3 ml of 6% perchloric acid solution at 2°. The homogenates were separated into acid-soluble and acid-insoluble fractions with a refrigerated centrifuge for 10 min at 10000 rpm and −2° routinly. The radioactivity of each fraction was measured with a liquid-scintillation counter (Nuclear Chicago, Co., Ltd.).

Results and Discussion

Four esters of I·2HCl used in the present experiments are given in Table I. The acyl derivatives were supposed to increase oil solubility and/or partition coefficient, and succinate was used to study the effects of increased water solubility on bioavailability.

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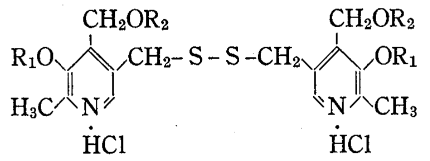
[7] K. Kitao, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), 25, 1335 (1977).

Table I. Pyrithioxin Dihydrochloride Derivatives



Compounds	Symbols	R_1	R_2	mol. wt.	mp
Pyrithioxin·2HCl	I·2HCl	H	H	441.4	184°
I-tetraacetate·2HCl	I–A·2HCl	CH_3CO	CH_3CO	609.5	159—160°
I-tetrapropionate·2HCl	1–P·2HCl	CH_3CH_2CO	CH_3CH_2CO	665.6	170—172°
I-tetra-*n*-butyrate·2HCl	I–B·2HCl	$CH_3CH_2CH_2CO$	$CH_3CH_2CH_2CO$	721.8	162—164°
I-4,4′-disuccinate·2HCl	I–S·2HCl	H	$HOOCCH_2CH_2CO$	687.6	153—154°

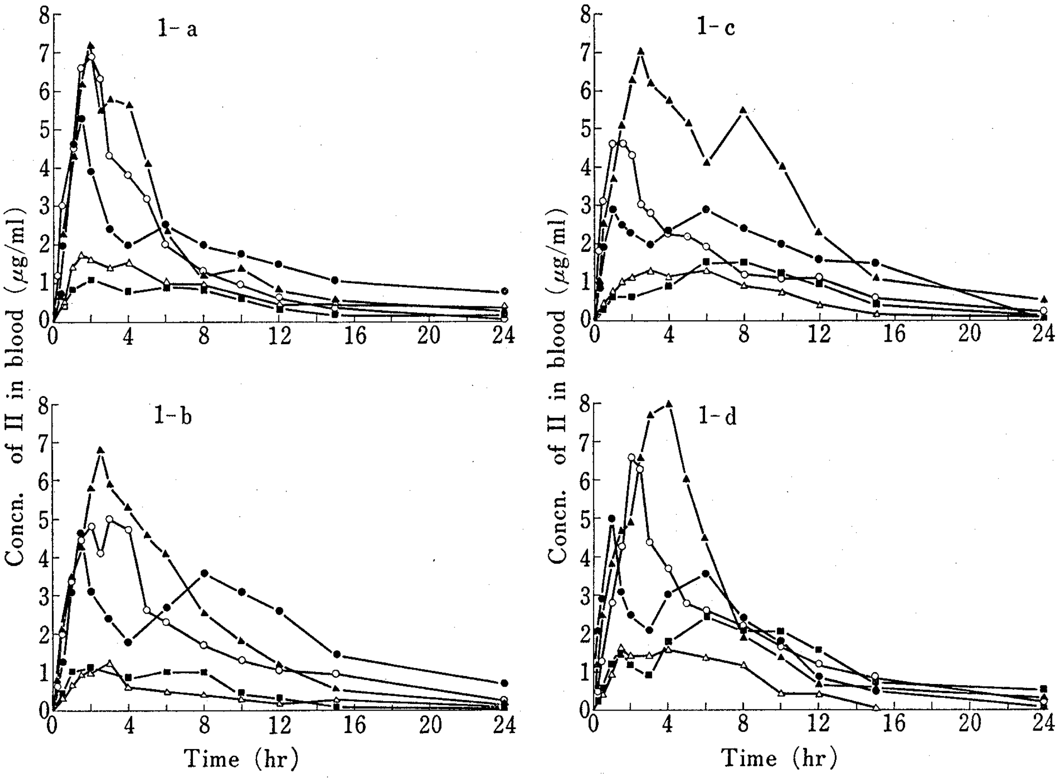


Fig. 1a—d. Blood Concentration of II after Single Oral Administration of I·2HCl and Its Esters in Four Dogs (Dose: Equivalent to 50 mg of I/kg)

key: ●, I·2HCl; ○, I–A·2HCl; ▲, I–P·2HCl; △, I–B·2HCl; and ■, I–S·2HCl

Blood Concentration

In the previous report,[1] it was presented that II is the only detectable metabolite in blood samples with HPLC following oral administration of I·2HCl. Thus, the blood concentration of II *versus* time curves (BCT) after single oral administration of I·2HCl and its esters for four dose were studied (Fig. 1, a—d). The figures were presented for the results of each dog. Apparent availabilities of I·2HCl and its esters after oral administration in terms of II were determined by a trapezoidal method. Thus, the area under the BCT curve (AUC) was calculated by resolving the area under the curve into small increment between the two adjacent plots and summing the incremental areas. The mean AUC values for four dogs with standard deviation were obtained for 0—8 and 0—24 hr periods (AUC_{0-8} and AUC_{0-24}). The values of mean peak blood concentration and mean peak time are also presented (Table II).

TABLE II. Mean Area under Curve, Peak Blood Concentration, and Peak Time after Single Oral Administration of I·2HCl and Its Esters to Four Dogs

Compounds	AUC_{0-8}[a], (μg·hr)/ml	AUC_{0-24}[a], (μg·hr)/ml	Peak blood concn., μg/ml	Peak time, hr
I·2HCl	21.2±2.0[b]	40.9± 6.0	4.45±1.07	1.25±0.29
I–A·2HCl	26.1±1.6[c]	37.4± 3.3	5.73±1.19	1.88±0.25[c]
I–P·2HCl	36.1±3.8[c]	52.3±11.5	7.25±0.53[c]	2.75±0.87[c]
I–B·2HCl	8.3±2.2[c]	12.9± 3.6[c]	1.45±0.24[c]	2.25±0.87[c]
I–S·2HCl	8.8±3.0[c]	17.3± 9.2[c]	1.55±0.67[c]	4.00±2.31[c]

[a] AUC was calculated by Trapezoidal Method.
[b] Values reported are mean ± SD for four dogs.
[c] Significantly different ($p<0.01$) from I·2HCl

It was interesting to note that the peak time of I–P·2HCl, when the blood level showed the heighest value of II among the five drugs, appeared significantly later than that of I·2HCl but the peak blood concentration of II for I–P·2HCl was 1.63 times higher than that of I·2HCl. The peak blood concentrations of I–B·2HCl and I–S·2HCl were significantly lower than that of I·2HCl being accompanied with small values of AUC_{0-8} and AUC_{0-24}. The AUC_{0-8} value of I–P·2HCl was 1.7 times larger than that of I·2HCl with 99% confidence. The AUC_{0-24} value of I–P·2HCl was appeared to be still larger than that of I·2HCl, but no statistically significant difference was obtained being subjected to large standard deviations.

Urinary Excretion

In the previous report,[1] it was confirmed that after oral and intravenous administrations of I to dogs, three metabolites, I–G, II, and II–G, were found in the urine, but not I. In a preliminary experiment, esters or I was not found in the urine after oral administration of esters. Thus, the esters are also excreted as the same metabolites of I in the urine after oral administration.

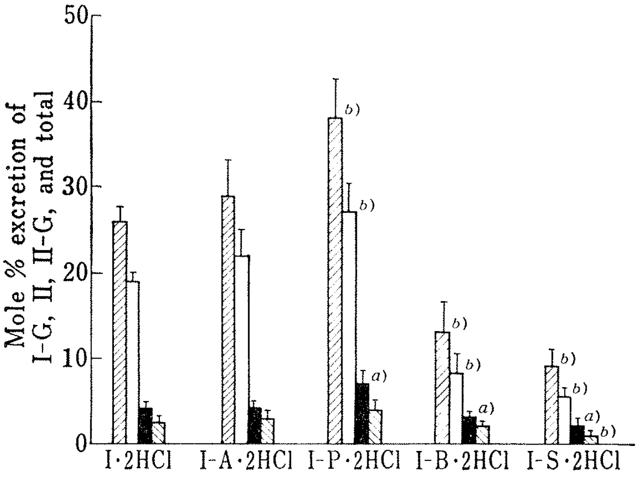


Fig. 2. Mole Percent Excretion of I–G, II, II–G, and Total in Urine after Single Oral Administration of I·2HCl and Its Esters to Four Dogs

Each bar represents the mean for four dogs, line indicates SD. a) indicates means that are significantly different ($p<0.05$) from I·2HCl, and b) indicates means that are significantly different($p<0.01$) from I·2HCl.
key: ▧, I–G; ☐, II; ■, II–G; and ▨, total,

TABLE III. Solubility of I and Its Esters in 1/30 M Phosphate Buff. (pH 6.5) at 37°

Compounds	Solubility, μM
I	19.1
I–A	58.7
I–P	25.4
I–B	3.8
I–S	$>1\times10^5$

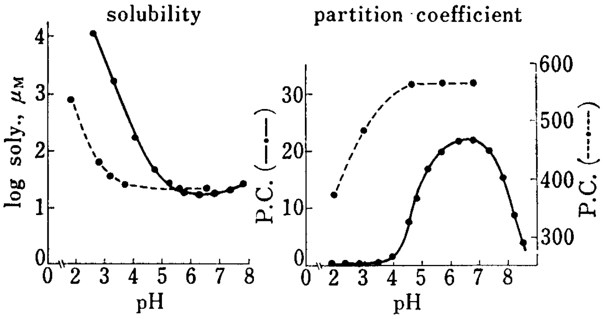


Fig. 3. pH-Profiles of Solubility and Partition Coefficient of I and I–P at 37°

key: —●—, I; and ---●---, I–P

Mean values of the cumulative amounts of each metabolite excreted in the urine and total excreted amounts in mole percent of dose were measured following single oral administration of each ester to four dogs (Fig. 2). A total excretion of I–P·2HCl increased by a factor of 1.47 with 99% confidence. No statistical difference with 95% confidence was noted between I–A·2HCl and I·2HCl. The values of I–B·2HCl and I–S·2HCl were significantly reduced. These results were closely correlated with those of AUC values.

pH-Profiles of Solubility and Partition Coefficient

To consider differences of urinary excretion and blood concentration of esters, pH-profiles of solubility and oil–water partition coefficient of I and I–P were measured (Fig. 3). The solubility of I and its esters at pH 6.5 and 37° was measured employing 1/30 M phosphate buffer[1] (Table III). The solubility of I–P at pH 6.5 was unexpectedly but slightly higher than that of I, and the partition coefficient of I–P was much larger than that of I at a pH range of 2—7. The pK_a values of I were reported 3.6 and *ca.* 10 with a solubility method,[8] and 4.43 and 8.84 with a spectral method[9] for the hydroxyl group and pyridine nitrogen, respectively. The ionization of I is considered to be quite similar to that of pyridoxine. α^4,3-*o*-Isopropylidene pyridoxine is a basic compound with a pK_a value of 5.53.[10] And 3,4′-diacylates of pyridoxine are also basic compounds and considered to have pK_a values around 5. Thus, inhibition of the resonance effect of the phenolic group markedly influences on pyridine ring and reduces the pK_a value of basic nitrogen of pyridoxine diacylates. A similar change is considered for the dissociation of the nitrogen of pyrithioxin tetraesters. Thus, the pH-profiles of solubility and partition coefficient of I–P were observed to change at pH around 4 with fairly constant values of the physicochemical properties at neutral and alkaline pH ranges. It seems that tetra-esters mainly exist as an unionized form in the intestinal tract. The solubility of tetra-esters decreased with an increase of the chain length of the acyl moiety. The water solubility of I–S was found to be more than 70 mg/ml at pH 6.5. But, its partition coefficient at pH 6.5 was 0.094.

Thus, it may be considered that the improved bioavailability of I–P·2HCl is ascribed to the significant increase in its partition coefficient with a slight increase in its water solubility at pH 6.5. Insufficient availabilities of I–B·2HCl and I–S·2HCl after oral administrations were considered to be responsible for the poor water solubility of 3.8 μM and small partition coefficient of 0.094 at pH 6.5, respectively.

Permeation through Everted Rat Intestine

Many pro-drugs having ester moiety have been reported to permeate through the intestinal tract after being hydrolyzed with regaining to their parent forms, *e.g.*, chloramphenicol palmitate,[11] and acylates of pyridoxine.[12]

To study the participation of hydrolysis with absorption processes *in vivo*, permeation experiments *in vitro* with everted rat intestine were performed following the modified method of Crane and Wilson[7] (Table IV.) It was found that the esters of I were easily hydrolyzed in the mucosal side by autogenous enzymes contained in intestinal fluid and/or intestinal tissue, I–B was completely hydrolyzed at 37° in 1 hr, but I–S was hydrolyzed only 20% at the same condition. In the serosal side, only I was found for all esters. Thus, it may be considered that after oral administration of I-esters, they are hydrolyzed by enzymes contained in the intestinal fluid and/or intestinal tissue before and/or during absorption. The parent form of I of esters are preferentially absorbed through the intestine and taken into the blood

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[10] N. Mizuno, Y. Iwayama, H. Takagi, and A. Kamada, *Yakuzaigaku*, **33**, 172 (1973).

[11] A.J. Glazko, W.H. Edgerton, W.A. Dill, and W.R. Lenz, *Antibiot. Chemotherapy*, **2**, 234 (1952).

[12] N. Mizuno, M. Aoki, A. Kamada, and S. Sumimoto, *Bitamin*, **38**, 125 (1968); N. Mizuno, M. Aoki, and A. Kamada, *Bitamin*, **38**, 129 (1968).

TABLE IV. Permeation of I and Its Esters through Everted Sac of Rat Intestine at 37° for 1 hr

Compound	Percent of I in mucosal side	Serosal side Concn. of I, μg/ml	Ester μg/ml
I	100	11.9±3.1[a]	—
I–A	75	10.7±3.1	0
I–P	91	12.0±3.0	0
I–B	100	17.8±4.8[b]	0
I–S	20	4.3±2.9[b]	0

a) Values reported are mean ± SD for 5 experiments.
b) indicates means that are significantly different($p<0.05$) from I.

stream. Thus, the poor availability of I–B is conclusively ascribed to its low solubility in intestinal fluid. The present result of I–B in the presence of 10% of propylene glycol in the mucosal side indicates that the availability of I–B can be increased to the extent of I–P by the enhancement of its solubility through the coadministration of solubilizing agents. The poor availability of I–S is ascribed to its low rate of hydrolysis in intestinal fluid being subjected to a possible inhibition of interactions between I–S and enzymes.

Transfer of ^{14}C-labelled Glucose through Blood-Brain Barrier

Quadbeck, *et al.*[13] studied the effect of oral administration of I on the passage of glucose through the blood-brain barrier in mice following intravenous administration of ^{14}C-labelled glucose. They found that under the influence of pyrithioxin, the lipoidal fraction of the brain is mainly affected by infusion of the radioactivity conveyed as glucose through the blood stream. It is therefore essential for the development of pro-drugs of pyrithioxin to analyse the glucose intake of the brain under the effect of the pro-drugs of I. They also found that repeated administration of I mainly affected an increase of radioactivity in the cerebrum, whereas the average values of the other sections of the brain little differed from the values obtained after a single administration of I. Thus, presently I and its ester derivatives were administered orally for 2 weeks, ^{14}C-labelled glucose was intraperitoneally administered to mice, and the brain samples were separated into the acid-insoluble fraction and acid-soluble fraction. The former fraction corresponds to the structural sections of brain tissue.

TABLE V. Incorporation of ^{14}C-Glucose into Mouse Brain after Oral Administration of I·2HCl and Its Esters

Compounds	Dose, mmole/kg	Acid soluble fraction dpm/mg brain	Acid insoluble fraction, dpm/mg brain
Control	—	1329±279[a]	810±222
I·2HCl	0.653	1527±184	1112±235[b]
I–S·2HCl	0.800	1505±183	1029±341
I–A·2HCl	0.505	1601±156	1030± 89[b]

a) Values reported are mean ± SD for 6 mice.
b) significantly different ($p<0.05$) from control

As a preliminary study, the effect of I–A·2HCl and I–S·2HCl on the glucose intake through the blood-brain barrier of mice was compared with that of I·2HCl (Table V).

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[13] G. Quadbeck, H.R. Landmann, W. Sachsse, and I. Schmidt, *Med. Exp.* **7**, 144 (1962).

Doses of the esters were tentatively determined from their availabilities of AUC and urinary recovery.

The acid-insoluble fractions of I·2HCl and I–A·2HCl were significantly increased with a confidence level of 95%. Whereas the dose of I–S·2HCl was much larger than that of I·2HCl and I–A·2HCl, but it failed to increase the uptake of glucose into the brain tissue. Thus, I–A·2HCl is considered to possess the pyrithioxin activity to the blood-brain barrier of mice. The present study has not covered I–P·2HCl but it may be considered that 1–P·2HCl has a similar or stronger activity than that of I·2HCl being subjected to the enhanced availability of ester.

Thus, it may be concluded that further comprehensive studies are required to elucidate relations between the drug activity of pro-drugs and their availability in terms of AUC and urinary recovery, but a chemical modification of I to pro-drugs seems to encourage for the clinical use of the drugs.

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