Effects of 4-Hydroxyantipyrine and Its 4-*O*-Sulfate on Antipyrine as Biodistribution Promoter

Yuhsuke Ohkawa, Miki Matsumura, Yuhji Kurosaki, Masateru Kurumi, Kenji Sasaki, and Taiji Nakayama*

Faculty of Pharmaceutical Sciences, Okayama University, 1–1–1 Tsushima-naka, Okayama 700–8530, Japan.
Received March 1, 2000; accepted January 13, 2001

The effects of 4-hydroxyantipyrine (4-OH), a major metabolite of antipyrine, and its 4-*O*-sulfate (4-S) on the pharmacokinetics of antipyrine were investigated in rats. Plasma elimination of intravenously administered antipyrine was significantly decelerated under a steady-state concentration of 4-OH but not under that of 4-S. Tissue-to-plasma concentration ratio (K_p) of antipyrine under its steady-state concentration was significantly increased in the brain and heart by the concomitant use of 4-OH, while similar use of 4-S had no effect. The enhancement of the blood-brain barrier (BBB) permeability of antipyrine caused by the concomitant use of 4-OH was believed to be concerned with the increase of the K_p value of antipyrine in the brain. These results suggested that 4-OH could be used as a biodistribution promoter.

Key words biodistribution promoter; antipyrine; 4-hydroxyantipyrine; 4-hydroxyantipyrine *O*-sulfate; concomitant use; drug/metabolite interaction

Considerable attention[1—4] has been focused on drug/drug interactions such as those in drug distribution, drug metabolism, drug absorption and receptor binding in multiple-drug therapy. In addition to drug/drug interactions, in the 1960s we reported the significance of drug/metabolite interaction(s) in the biopharmaceutical fate of a drug.[5—10] Studenberg and Brouwer also reported that the metabolites of phenobarbital inhibited the biotransformation of acetaminophen.[11] More recently we reported the effects of the concomitant use of *N*-acetyl-*p*-aminophenyl *O*-sulfate (APAPS)[12—14] and its geometric isomers.[15] In those reports, it was found that the tissue-to-plasma concentration ratio (K_p) of acetaminophen was significantly increased in the liver, heart, kidney and brain by the presence of APAPS but not by its geometric isomers. That is, APAPS seemed to play a role as a biodistribution promoter. This result prompted us to examine whether a similar drug/metabolite interaction could be observed when other drugs and metabolites were employed.

In this report, the possibility of 4-hydroxyantipyrine (4-OH),[16] which is one of the major metabolites of antipyrine, and its 4-*O*-sulfate (4-S) as a biodistribution promoter was examined by their concomitant use with antipyrine which is used as a bioactive drug.

MATERIALS AND METHODS

Materials 4-OH and 4-S (as ammonium salt) were prepared in our laboratory as below. Antipyrine and 4-S were dissolved in saline. 4-OH was dissolved in 10 mM NaOH containing saline before use. All chemicals and reagents were commercially available and of analytical grade or better.

Preparation of 4-OH This compound was prepared by the method of Hukki and Myry[16] to afford colorless needles (74%), mp 193—195 °C (lit.[16] 182 °C).

Preparation of 4-S To the solution of 4-OH (20 g, 0.10 mmol) in pyridine (300 ml) was added pyridine-sulfur trioxide complex (30 g, 0.19 mol) and the mixture was stirred at 0—10 °C for 48 h. After evaporation of the solvent of the reaction mixture in reduced pressure at 50 °C for 50 min. The resulting pale yellow viscous oil was dissolved in MeOH (150 ml). The ammonia gas was bubbled into the solution for 1.5 h and precipitated solid was filtered off. The filtrate was concentrated to *ca*. one-third under reduced pressure at 50 °C. Diethyl ether was then added to make the solution a milky suspension and allowed to stand in a refrigerator. The precipitated crystalline solid was collected by filtration to give 20 g (65%) of the titled compound as an ammonium salt which gave a single spot on tlc in several solvent systems. The structure of this compound was consistent with instrumental data and analysis data, mp 210—214 °C (decom.). *Anal*. Calcd for $C_{11}H_{15}N_3O_5S$: C, 43.85; H, 5.02; N, 13.95. Found: C, 43.61; H, 5.27; N, 13.79.

Animals Male Wistar rats (220—320 g) (Japan SLC, Inc., Hamamatsu, Japan) were used.

***In Vivo* Experiments** The rats were fasted overnight before use, but allowed free access to water, and were fixed in a dorsal position under urethane anesthesia (900 mg/kg, i.p.). Polyethylene tubing was cannulated into both the left femoral vein and the right femoral artery.

Pharmacokinetics of Antipyrine, 4-OH and 4-S: Antipyrine in saline (50 mg/kg), 4-OH in 10 mM NaOH–saline (50 mg/kg) and 4-S in saline (50 mg/kg) were respectively intravenously administered through the right femoral vein in a volume of 1 mg/kg, respectively. A blood sample was collected through the cannula fastened to the femoral vein at appropriate time intervals. Plasma samples were obtained by centrifugation at 10000×***g*** for 2 min, and all plasma samples were stored at −20 °C until analyzed.

Effect of 4-OH and 4-S on Plasma Concentration–Time Curves of Antipyrine: 4-OH and 4-S were intravenously administered at the initial dose (4.40 mg/kg and 6.13 mg/kg, respectively), followed by constant infusion (52.9 mg/kg/h and 44.2 mg/kg/h, respectively) to maintain the target plasma concentration (10 μg/ml and 50 μg/ml, respectively) through the cannula fastened to the left femoral vein throughout the experiment. Antipyrine (50 mg/kg) was intravenously administered under a steady-state concentration of 4-OH (or 4-S) 120 min after the start of infusion, and 10 min later, blood was continually collected from the right femoral artery. Saline was used as a control.

* To whom correspondence should be addressed. e-mail: nakayama@pheasant.pharm.okayama-u.ac.jp

Effect of Bolus Challenge with 4-OH and 4-S on Plasma Concentration Profile of Antipyrine: Antipyrine was intravenously administered at the initial dose (23.7 mg/kg), followed by a constant-rate infusion (24.1 mg/kg/h) to reach the target plasma concentration (100 μg/ml) through the cannula fastened to the left femoral vein throughout the experiment. 4-OH and 4-S (30 mg/kg and 100 mg/kg, respectively) were intravenously administered under a steady-state concentration of antipyrine at 1 h (for 4-OH and 4-S), 2 h (for 4-OH) and 3 h (for 4-S) after the start of infusion, respectively.

Effect of 4-OH and 4-S on Tissue-to-Plasma Concentration Ratio (K_p) of Antipyrine: 4-OH and 4-S (30 mg/kg and 100 mg/kg, respectively) were administered to the jugular vein at a steady-state of antipyrine (plasma concentration was 100 μg/ml). Five minutes after such administration, blood was collected from the abdominal aorta, and the rats were decapitated immediately. Tissue samples of the kidney, brain, liver, heart, spleen and lung were extirpated and homogenized (15000×***g***) in isotonic phosphate buffer (pH 7.4) for 5 min. Five milliliters of ethyl acetate was added to 1 ml of the homogenate and the mixture was shaken for 1 h. After the centrifugation (3000 rpm, 10 min and 4 °C), 4 ml of the upper layer was collected and evaporated *in vacuo*. The residue was dissolved in 300 μl of the mobile phase solvent for HPLC measurement. Saline was used as a control.

Effect of 4-OH and 4-S on Blood-Brain Barrier (BBB) Permeability of Antipyrine: 4-OH and 4-S were intravenously administered at the initial dose (4.40 mg/kg and 6.13 mg/kg, respectively), followed by constant infusion (52.9 mg/kg/h and 44.2 mg/kg/h, respectively) to maintain the target plasma concentration (10 μg/ml and 50 μg/ml, respectively) through the cannula fastened to the left femoral vein throughout the experiment. Antipyrine (50 mg/kg) was intravenously administered at a steady-state concentration of 4-OH (or 4-S) 120 min after the start of infusion. Five, 30, 60 or 120 min after the administration of antipyrine, the rats were decapitated. Tissue sample of the brain was treated as described in 'Effect of 4-OH and 4-S on Tissue-to-Plasma Concentration Ratio (K_p) of Antipyrine' and the resulting sample was supplied for HPLC measurement. Saline was used as a control.

In Vitro* Experiments** Effect of 4-OH and 4-S on Protein Binding of Antipyrine: To 450 μl of rat plasma preincubated at 37 °C for 2 min was added 50 μl of antipyrine in saline (final concentration was 20 μg/ml) and the mixture was incubated at 37 °C for 5 min. After incubation, the mixture was immediately ultrafiltrated through ULTRAFREE C3LGC (Japan Millipore Co., Tokyo, Japan). Centrifugation was continued for 20—30 min at 5000×g*** until 10—15% of the mixture was filtered. The filtrate was diluted with methanol (5-fold volume of the filtrate), and concentration of the free drug (unbound drug) was determined by HPLC measurement.

Effect of 4-OH and 4-S on Apparent Partition Coefficient of Antipyrine: A mixture of 4 ml of *n*-octanol saturated phosphate buffer solution (50 mM, pH 7.4) containing 0.1 mM of antipyrine and 0.1—1 mM of 4-OH (or 4-S) and 4 ml of *n*-octanol which was saturated with 50 mM phosphate buffer solution (pH 7.4) was severely shaken at room temperature for 30 min. The mixture was then slowly shaken at 37 °C for 2 h. The upper layer (*n*-octanol layer) was diluted with methanol and the aqueous layer was diluted with purified water. The concentrations of the test compounds were determined by HPLC measurement.

Analytical Method Antipyrine, 4-OH, and 4-S were simultaneously determined by the reversed-phase HPLC measurement according to our previous reports.[12,14] Briefly, a high performance liquid chromatograph (LC-10AD, Shimadzu, Kyoto, Japan) connected with an Inertsil ODS column (average particle size of 5 μm, 4.6 mm i.d.×250 mm, GL Science, Inc., Tokyo) and an UV detector (SPD-10A, Shimadzu, Kyoto) operated at 254 nm were used. A mixture of 50 mM phosphate buffer solution (pH 7.4) and methanol (75 : 25, v/v) was used as the mobile phase. The flow rate was 1.0 ml/min. Injection volumes were 30 μl, and the temperature was 40 °C. Five hundred microliters of methanol was added to 100 μl of plasma sample to denaturalize the protein. After precipitation of protein by centrifugation (3000 rpm× 10 min), 60 μl of the supernatant filtered through a 0.45 μm pore-size membrane filter (Japan Millipore Co., Yonezawa, Japan) was injected into HPLC for measurement. Antipyrine, 4-OH and 4-S were separately detected as symmetrical peaks.

Data Analysis Plasma concentrations (C_p) of antipyrine, 4-OH, and 4-S after the intravenous administration were fitted to a bi-exponential curve, $C_p = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t)$, by the nonlinear least-squares regression program[17] with a weight of $1/C_p$. Pharmacokinetic parameters were calculated from the estimated parameters, A, B, α and β, where the area under the plasma concentration–time curve (AUC) was calculated from $A/\alpha + B/\beta$ and the total body clearance, CL_{total}, was equal to Dose/AUC. The elimination half-life, $t_{1/2}\beta$, was calculated from $\ln 2/\beta$. The mean residence time, MRT, was calculated as $AUMC/AUC$, $AUMC$ meaning the area under the first moment curve. The volume of the central compartment, V_{d1}, was calculated as Dose/$(A+B)$. The apparent volume of distribution at steady-state, V_{dss}, was calculated as Dose$\cdot AUMC/AUC$[18,19] (Fig. 1).

Tissue-to-plasma concentration ratios (K_p) were estimated under the steady-state of antipyrine according to the following equations:

$$K_p = C_t/C_p = (C_t \cdot V_t - C_p \cdot V_e)/V_i/C_p$$

$$V_e = V_t \cdot \mathrm{IS}$$

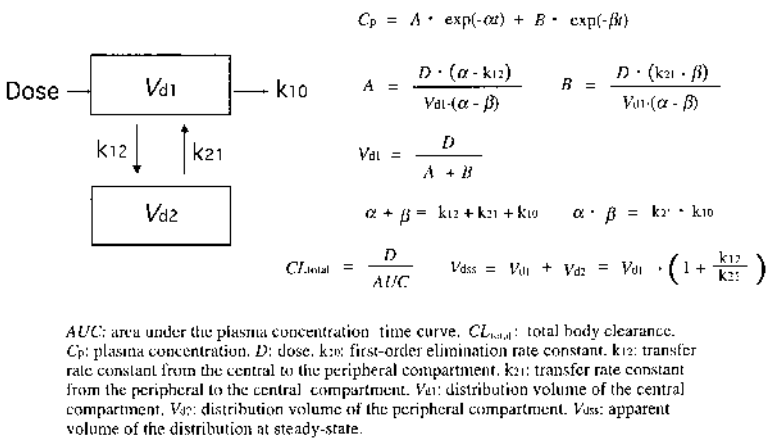
$$V_t = V_i + V_e$$

where C_t and C_p are the concentrations of antipyrine in the tissue and in the plasma, respectively; V_t is the tissue volume; V_i and V_e are the intracellular volume and the extracellular volume, respectively. The specific gravities of all tissues were assumed to be equal to 1. The K_p values of antipyrine corrected by inulin space are shown in Fig. 5.

To determine partition coefficient, the initial concentration of antipyrine in the aqueous layer and its concentration in the aqueous layer after equilibrium were estimated, and finally the concentration in the *n*-octanol layer after equilibrium was determined. The equation is shown in

$$P_{octanol} = C_{octanol}/C_{water} = (C_o - C_{water})/C_{water}$$

where $C_{octanol}$ is the concentration of the drug in the *n*-octanol layer after equilibrium; C_{water} is the concentration of the drug in the aqueous layer after equilibrium; C_o is the initial con-



AUC: area under the plasma concentration–time curve, CL_{total}: total body clearance, C_p: plasma concentration, *D*: dose, k_{10}: first-order elimination rate constant, k_{12}: transfer rate constant from the central to the peripheral compartment, k_{21}: transfer rate constant from the peripheral to the central compartment, V_{d1}: distribution volume of the central compartment, V_{d2}: distribution volume of the peripheral compartment, V_{dss}: apparent volume of the distribution at steady-state.

Fig. 1. Pharmacokinetic Model for Intravenous Administration

centration of the drug in the aqueous layer.

The permeability clearance of BBB (K_{in}) was determined according to the graphical evaluation method of Blasberg *et al.*[20] and Gjedde.[21] Usually the equation of mass balance is shown by

$$dC_{br}/dt = K_{in} \cdot C_p - K_{out} \cdot C_{br} \quad (1)$$

where C_{br} is the concentration in the brain, C_p is the concentration in plasma, K_{out} is the transport clearance from the brain to plasma. The efflux from the brain to plasma can be neglected ($C_{br} \ll C_p$) immediately following administration of the drug, so that Eqs. 2 and 3 can be derived from Eq. 1:

$$dC_{br}/dt = K_{in} \cdot C_p \quad (2)$$

$$C_{br}(T) = K_{in} \int_0^T C_p dt \quad (3)$$

Therefore, K_{in} can be obtained using the concentration in the brain at *T* (h) after administration of the drug ($C_{br}(T)$) and the area under the plasma concentration–time curve (*AUC*) until *T* (h) after administration of the drug ($\int_0^T C_p dt$). The single time point method[22] using Eq. 3, however, sometimes affords an error in the revision of the dose of the remaining drug in the vascular. On the other hand, the graphical evaluation method[20,21] using doses of the drug in the brain at several *T* (h) after its administration ($q_{tot}(T)$) is shown by

$$q_{tot}(T) = K_{in} \int_0^T C_p dt + V_{dbr} \cdot C_p(T) \quad (4)$$

where V_{dbr} is the total value of plasma volume in the brain and volume of extravascular compartment, $C_p(T)$ is the concentration in plasma at *T* (h) after administration of the drug. Eq. 5 can be derived from Eq. 4:

$$q_{tot}(T)/C_p(T) = K_{in} \int_0^T C_p dt / C_p(T) + V_{dbr} \quad (5)$$

From this equation, the regression line is obtained in which slope and intercept are K_{in} and V_{dbr}, respectively.

Statistical Analysis Statistical analyses of the pharmacokinetic parameters, effects of 4-OH and 4-S on plasma concentration–time curves of antipyrine, effects of bolus challenge with 4-OH and 4-S on plasma concentration profile of antipyrine, effects of 4-OH and 4-S on tissue-to-plasma concentration ratio (K_p) of antipyrine, effects of 4-OH and 4-S on protein bindings of antipyrine, effects of 4-OH or 4-S on apparent partition coefficient of antipyrine were performed by the Student's *t*-test and Tukey's multiple comparison procedure (ANOVA). In all statistical testing, *p* values less than 0.05 were considered to be statistically significant.

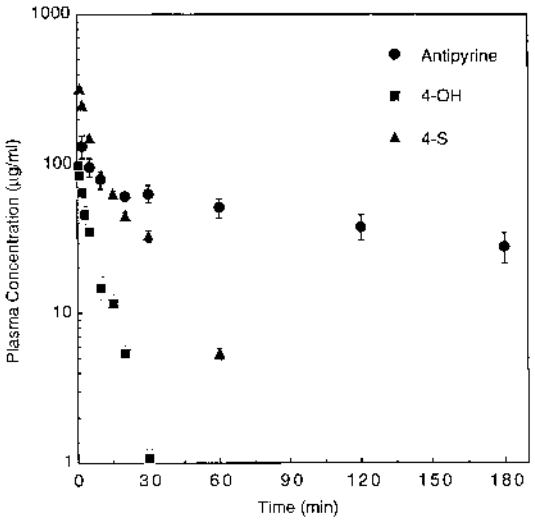


Fig. 2. Plasma Concentration–Time Curves of Antipyrine, 4-OH and 4-S (50 mg/kg, i.v.)

Results are expressed as the mean±S.E. (n=3—5).

RESULTS

Pharmacokinetics of Antipyrine, 4-OH and 4-S The plasma concentration–time curves of antipyrine, 4-OH and 4-S after intravenous administration (50 mg/kg, respectively) are shown in Fig. 2. Pharmacokinetics parameters of these compounds are summarized in Table 1. Compared with the parent compound (antipyrine), 4-OH and 4-S were quite rapidly eliminated from plasma, in the order of 4-OH, 4-S, and antipyrine. The CL_{total} values of 4-OH and 4-S were approximately 20 times and 3.5 times as large as that of antipyrine, respectively. Neither the administration of 4-OH nor the 4-S production of antipyrine from 4-OH or 4-S was recognized in plasma.

Effect of 4-OH and 4-S on Plasma Concentration–Time Curves of Antipyrine Plasma elimination profiles of intra-

Table 1. The Pharmacokinetic Parameters of Antipyrine, 4-OH and 4-S

Parameters	Antipyrine	4-OH	4-S
A (μg/ml)	148±46	73.4±0.3	281±4
B (μg/ml)	82.4±17.4	40.8±1.1	130±2
α (h^{-1})	18.1±1.3	23.9±0.6	22.1±1.2
β (h^{-1})	0.39±0.12	6.3±0.04	2.96±0.03
k_{12} (h^{-1})	10.6±1.4	5.7±0.1	8.8±0.8
k_{21} (h^{-1})	6.8±0.01	12.6±0.3	9.0±0.2
k_{10} (h^{-1})	1.02±0.23	12.0±0.03	7.2±0.1
V_{d1} (ml/kg)	235±65	438±3	122±1
V_{dss} (ml/kg)	610±209	635±13	242±16
CL_{total} (ml/h/kg)	254±119	5285±49	883±11

The dose of each drug was 50 mg/kg. Results are expressed as the mean±S.E. (n=3—5).

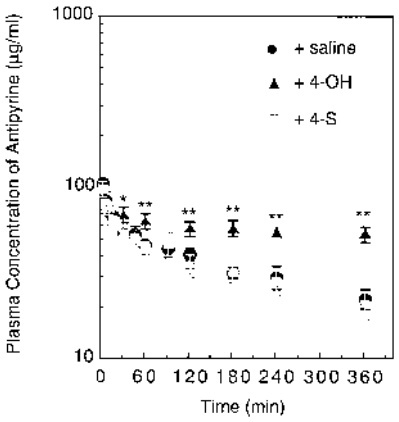


Fig. 3. Effects of 4-OH and 4-S on Plasma Concentration–Time Curves of Antipyrine (50 mg/kg, i.v.)

$*p<0.05$, $**p<0.01$; significantly different from control (saline). Results are expressed as the mean±S.E. (n=3—5).

venously administered antipyrine (50 mg/kg) under the steady-state concentration of 4-OH or 4-S are shown in Fig. 3. Concentrations of 4-OH and 4-S in plasma were kept at constant levels of about 10 μg/ml and 50 μg/ml, respectively. Plasma elimination of antipyrine was significantly decelerated by 4-OH, but was not affected by 4-S.

Effect of Bolus Challenge with 4-OH and 4-S on Plasma Concentration Profile of Antipyrine The effect of intravenously administered 4-OH (30 mg/kg) and 4-S (100 mg/kg) on steady-state plasma concentration of antipyrine is shown in Fig. 4. The plasma concentration of antipyrine was drastically decreased by the intravenous coadministration of 4-OH or 4-S. However, this phenomenon was transient and the plasma concentration of antipyrine was restored to the original level within 10—40 min.

Effect of 4-OH and 4-S on Tissue-to-Plasma Concentration Ratio (K_p) of Antipyrine The K_p values of antipyrine 5 min after the intravenous bolus administration of 4-OH or 4-S under the steady-state concentration of antipyrine are shown in Fig. 5. These K_p values were increased significantly in the brain and heart by the administration of 4-OH, but were not affected by 4-S.

Effect of 4-OH and 4-S on Protein Binding of Antipyrine As shown in Fig. 6, approximately 15% of antipyrine was bound to plasma protein. This was reduced with the addition of 4-OH.

Effects of 4-OH and 4-S on Apparent Partition Coefficient of Antipyrine Apparent partition coefficient of antipyrine in the presence of 4-OH (or 4-S) is shown in Fig. 7. This partition coefficient increased with the addition of 4-OH until three-fold 4-OH was added, however, it decreased with the addition of more than three-fold 4-OH. In contrast, the partition coefficient of antipyrine decreased with the addition of only one-fourth-fold 4-S, and no further change in this value was observed by a rise in this concentration.

Effects of 4-OH and 4-S on Blood-Brain Barrier (BBB) Permeability of Antipyrine The concentration of antipyrine in the brain after its intravenous administration under the steady-state plasma concentration of 4-OH or 4-S was

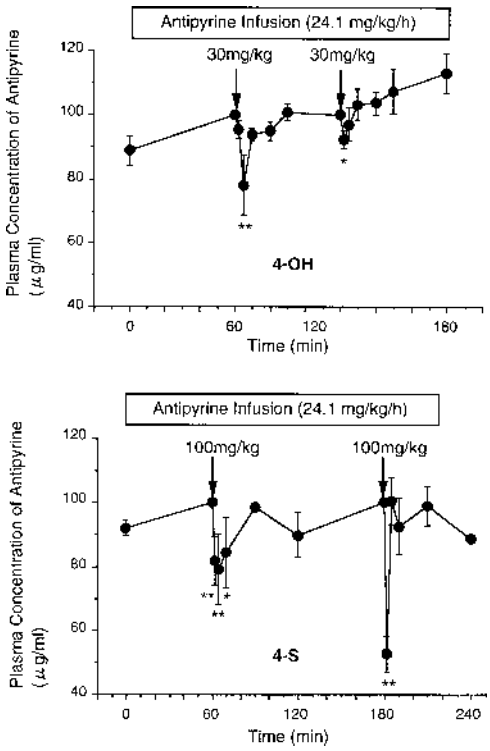


Fig. 4. Effect of Bolus Challenge with 4-OH and 4-S on Plasma Concentration Profile of Antipyrine

$*p<0.05$, $**p<0.01$; significantly different from plasma concentration before bolus challenge with 4-OH and 4-S. Results are expressed as the mean±S.E. (n=4).

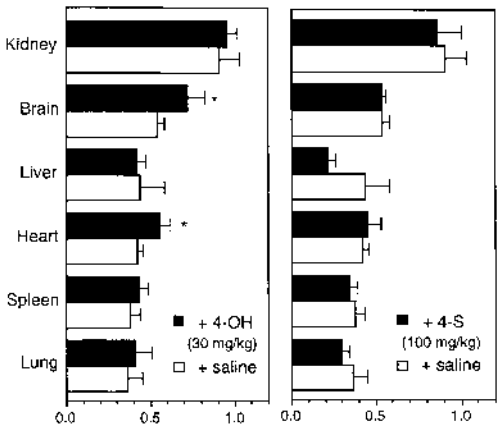


Fig. 5. Effect of 4-OH and 4-S on Tissue-to-Plasma Concentration Ratio (K_p) of Antipyrine

$*p<0.05$; Significantly different from control (saline). Results are expressed as the mean±S.E. (n=4).

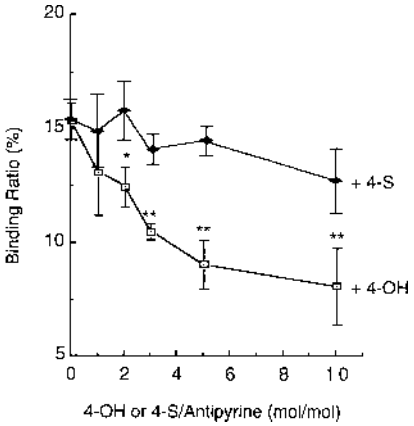


Fig. 6. Effect of 4-OH and 4-S on Protein Bindings of Antipyrine

$*p<0.05$, $**p<0.01$; Significantly different from bound ratio without 4-OH and 4-S. Results are expressed as the mean±S.E. ($n=3$).

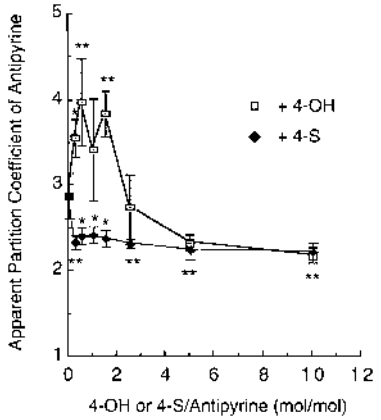


Fig. 7. Effect of 4-OH or 4-S on Apparent Partition Coefficient of Antipyrine

$*p<0.05$, $**p<0.01$; Significantly different from apparent partition coefficient of antipyrine without 4-OH and 4-S. Results are expressed as the mean±S.E. ($n=3$).

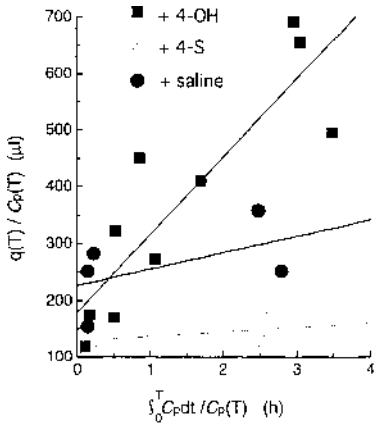


Fig. 8. Effect of 4-OH and 4-S on Blood-Brain Barrier (BBB) Permeability of Antipyrine

determined and plotted according to the graphical evaluation method.[20,21] The result is shown in Fig. 8. The permeability clearance of BBB (K_{in}) and the total distribution volume (V_{dbr}) are summarized in Table 2. K_{in} of antipyrine under the steady-state plasma concentration of 4-OH was 137 μl/min/g, which was about 5 times as large as that of the control. But

Table 2. The Permeability Clearance of BBB (K_{in}) and The Total Distribution Volume (V_{dbr}) in Brain Determined by The Graphical Evaluation Method

Condition	K_{in} (μl/min/g)	V_{dbr} (μl/g)
Control	29.1	226
+4-OH	137	179
+4-S	7.56	129

Graphical evaluation method using doses of the drug in the brain at several T (h) after administration of the drug ($q_{tot}(T)$), shown in $q_{tot}(T)=K_{in}\int_0^T C_p dt+V_{dbr}\cdot C_p(T)$ (Eq. 1), where K_{in} is the permeability clearance of BBB, $\int_0^T C_p dt$ is the area under the plasma concentration–time curve until T (h) after the administration, V_{dbr} is the total value of plasma volume in the brain and volume of the extravascular compartment, $C_p(T)$ is the concentration in plasma at T (h) after the drug administration. Equation 2 can be derived from the Eq. 1: $q_{tot}(T)/C_p(T)=K_{in}\int_0^T C_p dt/C_p(T)+V_{dbr}$(Eq. 2). From this equation, the regression line is obtained in which slope and intercept are K_{in} and V_{dbr}, respectively (see Fig. 8).

K_{in} of antipyrine under the steady-state plasma concentration of 4-S was 7.56 μl/min/g, which was about one-fourth that of the control. V_{dbr} was decreased by the concomitant administration of either 4-OH or 4-S.

DISCUSSION

We have already demonstrated that the pharmacokinetics of drugs are sometimes influenced by the biopharmaceutical interactions with their metabolites.[5—10,12—14] In this report, the effects of the metabolite of antipyrine (4-OH) and its sulfate (4-S) on the pharmacokinetics of antipyrine which was the centrally acting drug were investigated in rats.

4-OH under a steady-state concentration significantly decelerated the plasma elimination profile of intravenously administered antipyrine, while 4-S did not affect it. (see Fig. 3). Similarly, the K_p value of antipyrine increased in the brain and heart by the concomitant use of 4-OH but was not affected by 4-S (see Fig. 5). It is therefore possible that 4-OH inhibited the metabolism of antipyrine, thus increasing its plasma concentration and enhancing its transition to tissues as a result. The plasma concentration of antipyrine was drastically decreased by the intravenous bolus coadministration of 4-OH (or 4-S) at steady-state plasma concentration of antipyrine, and this phenomenon was stronger when 4-OH was used than 4-S (see Fig. 4). It seems that this decrease was caused by the transition of antipyrine to tissues.

In general, an intravenously administered drug must pass through the plasma membrane for distribution to the tissues, and one of the factors allowing membrane transport are the protein binding ratio and partition coefficient of the drug. The protein binding ratio of antipyrine was reduced by the concomitant use of 4-OH (see Fig. 6) in this study. But, considering the distribution volume of antipyrine (235±65 ml/kg for V_{d1} and 610±209 ml/kg for V_{dss}, see Table 1), the increase of unbound antipyrine in plasma by 4-OH seemed to make a relatively small contribution to the enhancement of the transition to tissues.

The partition coefficient of antipyrine was apparently increased by the concomitant use of 4-OH (see Fig. 7). One of the effects changing the partition coefficient of the compound is the salting-out effect. At first, we speculated that this effect caused the increase of the partition coefficient of antipyrine, but 50 mM phosphate buffer solution was employed in this experiment, and the concentration of antipyrine and 4-OH

were only 0.10 mM and 0.025—1.0 mM, respectively. That is, the salt concentration of the buffer solution was 50—2000-fold those of antipyrine and 4-OH. It would seem very difficult for the migration of a small amount of 4-OH from the water layer to the organic layer to have this effect on the partition coefficient of antipyrine under our conditions. This factor of the salting-out effect was thus neglected, and at present it is not clear why the partition coefficient of antipyrine was increased with the concomitant use of 4-OH. Furthermore, if the change in this coefficient were to cause an enhancement in its K_p value, it seems that K_p values would increase in all organs; this was not the result, however. We therefore believe that there was some kind of interaction between antipyrine and 4-OH, and that this interaction increased the partition coefficient of antipyrine and also specifically caused the enhancement of its K_p value in the brain and heart. At present, however, we have no plausible explanation of what kind of interaction this was or why this phenomenon occurred.

The concentration of antipyrine in the brain after its intravenous administration at a steady-state plasma concentration of 4-OH or 4-S was determined (see Fig. 8), and the K_{in} value at the concentration of 4-OH was about 5 times as large as that of the control. V_{dbr} value was decreased by the concomitant administration of either 4-OH or 4-S (see Table 2). From these results, it seems that the enhancement of the BBB permeability of antipyrine caused by 4-OH resulted in the increase of its K_p value in the brain.

Further study on the mechanism of the distribution promotion effect will be performed in the near future.

CONCLUSION

The K_p values of antipyrine increased significantly in the brain and heart with the concomitant administration of 4-OH under the steady-state concentration of antipyrine in plasma, but were not affected by 4-S. On the other hand, the partition coefficient of antipyrine was apparently increased by the concomitant use of 4-OH. It seemed this phenomenon would be concerned with a mechanism to increase the K_p values of antipyrine in the brain and heart. However, it was not clear what kind of interaction between antipyrine and 4-OH acted to enhance the partition coefficient of antipyrine. The protein binding ratio of antipyrine seems to make relatively small contribution to the enhancement of its transition to tissues.

The enhancement of the BBB permeability of antipyrine caused by 4-OH was believed to be concerned with increase of the K_p value of antipyrine in the brain. Recently various methods such as the BBB basic amine transporter, which is used for the transition of hydrophilic and charged compounds to the brain,[23] and nerve growth factor which is modified with polyamine[24] have been suggested as the drug delivery system to the brain. Increase of BBB permeability by stress exposure has also been reported.[25] From another viewpoint, increase of drug transition to the brain by the concomitant use of 4-OH as recognized in this study is information useful for application of a drug combination as a biodistribution promoter.

Acknowledgment We are grateful to The SC-NMR Laboratory of Okayama University for 200 MHz ^{1}H-NMR experiments.

REFERENCES

1) Niwa H., Nakayama T., *Arch. Pract. Pharm.*, **30**, 206—211 (1970).
2) Thomas B. H., Zeitz W., Coldwell B. B., *J. Pharm. Sci.*, **63**, 1367—1370 (1974).
3) Price V. F., Gale G. R., *Res. Commun. Chem. Pathol. Pharmacol*., **57**, 249—260 (1987).
4) Tsuji A., Sato H., Okazaki E., Nagata O., Kato H., *Biochem. Pharmacol*., **37**, 4408—4411 (1988).
5) Niwa H., Nakayama T., *Yakugaku Zasshi*, **88**, 535—541 (1968).
6) Niwa H., Nakayama T., *Yakugaku Zasshi*, **88**, 542—548 (1968).
7) Niwa H., Nakayama T., *Yakugaku Zasshi*, **88**, 843—846 (1968).
8) Nakayama T., *Yakugaku Zasshi*, **88**, 847—851 (1968).
9) Niwa H., Nakayama T., *Bull. Tohoku Pharmaceutical Collage*, **16**, 1—13 (1969).
10) Niwa H., Nakayama T., *Bull. Tohoku Pharmaceutical Collage*, **16**, 15—23 (1969).
11) Studenberg S. D., Brouwer, K. L. R., *Pharmacok. Biopharm*., **21**, 175—194 (1993).
12) Nakayama T., Sawamoto T., Kurosaki Y., Kimura T., *Int. J. Pharmaceut*., **121**, 55—63 (1995).
13) Sawamoto T., Kurosaki Y., Kimura T., Nakayama T., *Biol. Pharm. Bull*., **19**, 77—82 (1996).
14) Sawamoto T., Kurosaki Y., Sasaki K., Kimura T., Nakayama T., *Int. J. Pharmaceut*., **146**, 181—191 (1997).
15) Nakayama T., Sawamoto T., Karino T., Matsumura M., Sasaki K., Kurosaki Y., Kimura T., *Biol. Pharm. Bull*., **20**, 522—529 (1997).
16) Hukki J., Myry J., *Acta Chem. Scand*., **13**, 174—177 (1959).
17) Yamaoka K., Tanigawara Y., Nakagawa T., Uno T., *J. Pharmacobio-Dyn*., **4**, 879—885 (1981).
18) Etagere M., Nakamura H., Maruyama T., Imamura Y., Takadate Y., *Chem. Pharm. Bull.*, **37**, 498—501 (1989).
19) Yamaoka K., Nakagawa T., Uno T., *J. Pharmacokinet. Biopharm*., **6**, 547—558 (1978).
20) Blasberg R. G., Fenstermacher J. D., Patlak C. S., *J. Cereb. Blood Flow Metab*., **3**, 8—32(1983).
21) Gjedde A., *J. Neurochem*., **36**, 1463—1471 (1981).
22) Ohno K., Pettigrew K. D., Rapoport S. I., *Am. J. Physiol*., **235**, 299 (1978).
23) Traci L. M., David E. B., Alvin V. T., Jr., Warren J. B., Chris R. M. David D. A., *Neurosci. Let*., **258**, 25—28 (1998).
24) Joseph F. P., Geoffry L. C. Jagjit S. G., *J. Neurochem*., **71**, 1651—1660 (1998).
25) Ivana S., Dmitrij T., Daniela J., *Brain Res. Bull.*, **45**, 175—178 (1998).