Pharmacokinetic Drug Interactions between Ampiroxicam and Sulfaphenazole in Rats

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Received September 18, 1998; accepted November 20, 1998

The aim of the present study was to determine the effect of sulfaphenazole (SP) on the pharmacokinetics of ampiroxicam (AM) which is metabolized by cytochrome P-450 (CYP) 2C9, since SP is a potent inhibitor of CYP 2C9, and so a dramatic pharmacokinetic drug interaction between both drugs is assumed after dosing. Single intravenous and oral administrations of AM (5 and 7.5 mg/kg piroxicam equivalent, respectively) and SP (80 and 120 mg/kg, respectively) to rats did not significantly alter the elimination kinetics of AM and piroxicam (PX) converted from AM. When SP was preloaded orally at 2 h before the dosing of AM, and when AM and SP were orally coadministered for 7 d, the elimination of PX from plasma was slightly retarded and the area under the plasma concentration–time curve (AUC) was increased 77 and 53%, respectively, but not significantly, compared with those after AM alone. On the other hand, a significantly decreased metabolic conversion of PX to 5'-hydroxy-PX in plasma was observed by these treatments ($p < 0.05$).

In order to clarify the mechanism for the interaction, hepatic and intestinal metabolizing enzyme activities, CYP, uridine 5'-diphosphoglucuronyltransferase (UDPGT) and aryl esterase, were assayed after single and multiple oral administrations of AM or AM and SP. The enzyme activities were hardly inhibited by the treatment, indicating that the inhibition of CYP and hydrolytic enzymes by SP was approximately denied. These results suggest that SP does not significantly affect the pharmacokinetics of AM and PX in rats. However, the pharmacokinetic drug interaction between both drugs in man may not always be ignored.

Key words ampiroxicam–sulfaphenazole interaction; pharmacokinetic interaction; metabolic conversion

Ampiroxicam (AM), an ethoxycarbonyl ether prodrug of piroxicam (PX), is a non-steroidal anti-inflammatory drug (NSAID) which has been developed with the intention of diminishing gastrointestinal irritation; it is used in the treatment of patients with rheumatological disorders, osteoarthritis and other inflammatory disorders. In man, AM is completely and rapidly converted to PX by nonspecific esterases in the intestinal wall.¹⁾ The pharmacokinetics of PX from AM in man are essentially the same as that after PX itself, except that the maximum plasma concentration ($C_{\max}$) is slightly lower and the time ($t_{\max}$) to $C_{\max}$ is slightly longer after the administration of AM.²⁾ Despite many reports on the biological activity of AM in rats,^{3,4)} few studies have attempted to characterize the pharmacokinetics of the drug in this animal. The disposition pattern of AM in rat thus remains largely undefined.

Numerous pharmacokinetic interactions between drugs have been documented in humans. However, drug interactions caused by AM or PX are relatively few, although there are some reports on the interactions involved in PX and cimetidine in man⁵⁾ and rat.⁶⁾ PX is extensively metabolized by cytochrome P-450 (CYP) 2C9 in man,⁷⁾ and sulfaphenazole (SP) is a potent inhibitor of CYP2C9.⁸⁾ Since the main metabolic pathway of PX is hydroxylation, a reaction generally dependent on the hepatic CYP system, PX is potentially susceptible to interventions that alter liver metabolic activity, such as concurrent cimetidine administration. Thus, the interaction of PX and other drugs that affect the metabolic pathway of PX is of major clinical importance.

The aim of the present study was to examine potential interactions between AM or PX and other drugs using rats. The

influence of SP on the pharmacokinetics of PX, the metabolism of PX to 5'-hydroxypiroxicam (OH-PX), and the hepatic and intestinal drug metabolizing enzyme activities were estimated to clarify the possible mechanism of the pharmacokinetic drug interaction.

MATERIALS AND METHODS

Materials AM and OH-PX were generous gifts of Toyama Chemical Industrials Co. (Tokyo, Japan) and Pfizer Pharmaceutical Co. (Groton, CT, U.S.A.), respectively. PX and SP were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Naproxen, an internal standard for high performance liquid chromatography (HPLC), was obtained from Wako Pure Chemical Industries Co. (Osaka, Japan). Uridine 5'-diphosphoglucuronate was obtained from Nacalai Tesque Co. (Kyoto, Japan). All other chemicals and solvents used were of reagent grade or HPLC quality.

Treatment of Animals Male Wistar rats (SLC, Inc., Shizuoka, Japan), weighing 200–230 g, were used throughout this study. The animals were maintained on an MF diet (Oriental Yeast Co., Japan) for 3–4 d prior to the experiment. A group of 3 or 5 rats was randomly selected from the population. On the day before the experiment, the rat jugular vein was cannulated with silicone tubing.⁹⁾

Single Intravenous (i.v.) Administration: One group was treated with a single i.v. administration of AM (5 mg/kg, PX equivalent) dissolved in dimethyl sulfoxide (DMSO). The second group was treated with a single i.v. administration of AM and SP (80 mg/kg) as a DMSO solution.

Single and Multiple Oral Administrations: AM (7.5

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mg/kg, PX equivalent) was administered orally as an aqueous suspension (1.0 ml/250 g) in 1.5% carboxymethylcellulose (CMC) to the rat fasted for 12 h before the experiment. The rats of the second group were treated with a single oral administration of AM and SP (120 mg/kg) as a CMC suspension (coadministration group). The third group of rats received SP (120 mg/kg) and at 2 h after SP, AM (7.5 mg/kg, PX equivalent) was administered orally (preloaded group). In some experiments on enzyme activities, SP alone (120 mg/kg) was orally administered to rats. In multiple oral administration studies, animals were treated with daily oral administrations of AM (7.5 mg/kg, PX equivalent) or AM and SP (120 mg/kg) for 7 d, and had free access to MF diet 5 to 10 h after each drug dosing (multiple dose group).

Assay of Ulcerogenic Activity¹⁰⁾ The ulcerogenic activities were estimated after the multiple oral administration of AM, SP or AM and SP. At 12 h after the final dosing of the drug(s) or CMC (control), 2% HCHO (10 ml) was poured into the stomach and intestine of the rat using an injector *in situ*. The stomach and intestine were removed from the rats and opened on the board. The lesions on the gastrointestinal mucosa were counted by visual examination using a 2×2 binocular magnifier, with all the ulcers >0.5 mm being recorded.

Protein Binding Study Blood samples at 1 h after i.v. dosing of AM or AM and SP were drawn from the rats and the plasma was separated immediately by centrifuging. The plasma was subjected to centrifugation at 3000 rpm for 20 min at an ambient temperature through an ultrafree membrane (MPS-1, Amicon Div., Danvers, MA, U.S.A.). The concentrations of PX in the ultrafilters were determined by the HPLC assay as described hereafter.

Preparation of Hepatic 9000×g Supernatant and Intestinal Mucosa Suspension Animals were fasted for 12 h prior to the experiment. The livers were perfused through the portal vein with ice-cold 0.9% NaCl solution and excised. The 9000×g supernatant was prepared by the ordinary method. Intestinal contents were removed by flushing with saline, and the mucosa of a 5 cm length of duodenum was scraped off with a slide glass. The mucosa was suspended in 5.0 ml of 0.85% NaCl–10 mM phosphate buffer, pH 7.4, using a glass homogenizer.

Protein Determination Protein concentration was determined by the method described by Lowry *et al.*¹¹⁾ with bovine albumin, fraction V, as a standard.

Enzyme Assay The concentration of CYP in the 9000×g supernatant was determined by the method of Omura and Sato.¹²⁾ The activity of aryl esterase was determined by the method of Beaufay *et al.*¹³⁾ Uridine 5'-diphosphoglucuronyltransferase (UDPGT) activity was estimated using *p*-nitrophenol as a substrate according to the method described by Mulder.¹⁴⁾

Determination of AM, PX and OH-PX Blood samples (0.2 ml) were collected periodically after dosing through the tubing. The plasma was separated immediately by centrifuging and stored frozen until the time of assay. AM and PX in plasma (100 µl) were determined by the method of Kanazawa *et al.*,¹⁵⁾ except that naproxen was used (25 or 50 µg/ml methanol) as the internal standard for the HPLC method. OX-PX was assayed by the method of Richardson and Ross.¹⁶⁾

Fig. 1. Plasma Concentration–Time Profiles of AM and PX after i.v. Administration of AM Alone and in Combination with SP

Each point represents the mean $\pm$ S.D. ($n=3-5$). Dose: AM, 5 mg PX equivalent/kg; SP, 80 mg/kg. $\square$, AM after AM alone; $\blacksquare$, AM after AM+SP; $\circ$, PX after AM alone; $\bullet$, PX after AM+SP.

Pharmacokinetic and Statistical Analysis Kinetic parameters were calculated by using the least-squares fit program, MULTI.¹⁷⁾ The plasma concentration–time data after i.v. and oral administrations were analyzed by the noncompartment method.

The area under the plasma concentration–time curve (*AUC*) up to the last sampling point was calculated by the trapezoidal method, and the *AUC* beyond the last observed plasma concentration (C_n) was extrapolated according to C_n/β (or k_e , the elimination rate constant). The pharmacokinetic parameters (k_e and *Cl*) were calculated from the usual equations.¹⁸⁾ The area under the first moment curve (*AUMC*) and the mean residence time (*MRT*) were calculated by means of moment analysis.¹⁸⁾ The absolute bioavailability was calculated using the *AUC* values.

The means of all data are presented with their standard deviation (mean $\pm$ S.D.). Statistical analysis was performed using an unpaired Student's *t*-test, and the significance level adopted was $p < 0.05$.

RESULTS

Plasma Concentration after i.v. Administration The mean plasma concentrations of AM and PX after a single i.v. administration of AM alone or in combination with SP are depicted in Fig. 1. The plasma levels of AM and PX converted from AM declined approximately in a biexponential manner. However, the disposition of AM from plasma was highly rapid compared with that of PX, suggesting that AM was rapidly and completely converted to PX in the circulation system. The coadministration with SP had no effect on the elimination of AM and PX, but the initial concentrations of PX converted after the dosing of AM alone were slightly higher than those for the coadministration group. The pharmacokinetic parameters calculated from the data are shown in Table 1, with the plasma protein binding data. PX showed a relatively long elimination half-life in rats, although these values were smaller than those in man (37.5–60.4 h).^{19–22)} OX-PX was not determined because there was no necessity for an assay.

The presence of SP did not affect the plasma protein bind-

Table 1. Pharmacokinetic Parameters of AM and PX after i.v. Administration of AM Alone and in Combination with SP

Parameter	AM alone	AM+SP	AM alone	AM+SP
	PX		AM	
AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)	155.09 ± 62.60	174.12 ± 51.82	2.79 ± 1.01	2.27 ± 0.80
MRT (h)	7.72 ± 1.77	8.38 ± 2.87	0.20 ± 0.18	1.08 ± 0.27
k_e (h^{-1})	0.55 ± 0.66	0.13 ± 0.04	48.44 ± 12.01	63.74 ± 0.51
$t_{1/2}$ (h)	3.24 ± 2.90	5.70 ± 1.84	0.01 ± 0.00	0.010 ± 0.00
Cl ($\text{ml}/\text{h}/\text{kg}$)	36.13 ± 12.11	29.52 ± 12.31	1792.10 ± 217.82	2460.91 ± 1094.13
Protein binding (%) ^{a)}	97.4 ± 1.6	97.7 ± 0.6		

Dose: AM, 5 mg PX equivalent/kg; SP, 80 mg/kg. Each value represents the mean $\pm$ S.D. ($n=3-5$). a) Protein binding (%) of PX in plasma 1 h after i.v. dosing.

Table 2. Pharmacokinetic Parameters of PX after Single Oral Administration of AM Alone and in Combination with SP

Parameter	AM alone	AM+SP	SP preloaded	AM alone	AM+SP	SP preloaded
	PX			OH-PX		
AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)	216.76 ± 44.48	258.33 ± 62.87	382.66 ± 147.10	22.57 ± 4.24	15.22 ± 6.13	19.99 ± 6.97
MRT (h)	10.66 ± 2.49	10.68 ± 1.62	10.78 ± 3.24	14.52 ± 2.44	13.78 ± 5.71	17.21 ± 6.78
k_e (h^{-1})	0.11 ± 0.03	0.10 ± 0.01	0.10 ± 0.07	0.07 ± 0.01	0.11 ± 0.07	0.07 ± 0.03
$t_{1/2}$ (h)	6.63 ± 1.33	7.38 ± 1.12	7.45 ± 2.24	10.28 ± 2.19	9.10 ± 5.23	11.89 ± 4.68
$C_{\max}$ ($\mu\text{g}/\text{ml}$)	16.98 ± 2.96	17.75 ± 0.27	20.87 ± 4.26	1.41 ± 0.22	$1.04 \pm 0.11^a)$	$1.03 \pm 0.10^a)$
Bioavailability (%)	93.2 ± 19.1	111.0 ± 27.0	164.5 ± 63.2	—	—	—
$AUC_{\text{OH-PX}}/AUC_{\text{PX}}$	—	—	—	0.11 ± 0.01	0.06 ± 0.02	$0.06 \pm 0.01^a)$

Dose: AM, 7.5 mg PX equivalent/kg; SP, 120 mg/kg. Each value represents the mean $\pm$ S.D. ($n=4-5$). a) $p < 0.05$ compared with AM alone.

Fig. 2. Plasma Concentration-Time Profiles of PX (A) and OH-PX (B) after Single Oral Administration of AM Alone and in Combination with SP

Each point represents the mean $\pm$ S.D. ($n=4-5$). Dose: AM, 7.5 mg PX equivalent/kg; SP, 120 mg/kg; $\circ$, PX and OH-PX after AM alone; $\bullet$, PX and OH-PX after AM+SP; $\blacktriangle$, PX and OH-PX after preloading of SP. a) $p < 0.05$ compared with AM alone.

ing of PX, which bound to plasma protein to a high degree (unbound fraction (f_u), 0.026) (Table 1).

Plasma Concentration of PX after Oral Administration
Concurrent Administration of SP: The mean plasma concentration-time curves for PX converted from AM after a single oral administration of AM alone or in combination with SP are shown in Fig. 2. No parent drug was detected in plasma after the oral administration, suggesting the complete conversion of AM to PX by first-pass metabolism. The coadministration with SP increased the plasma levels of PX only slightly, but not significantly, compared with those after AM alone, consequently conferring about a 19% increase in the AUC (Table 2). Additionally, there were no significant differences between the kinetic parameters of both groups. The bioavailability of PX after the oral administration of AM was

$93.2 \pm 19.1\%$.

Preloading and Multiple Dosing with SP: When SP was preloaded prior to the administration of AM, significantly higher plasma levels of PX were partly observed compared with those after AM alone, with 1.8 times larger AUC and 1.2 times higher $C_{\max}$ than those after AM alone, probably due to the decreased metabolic conversion of PX. However, no significant difference between the parameters of either group was observed in this experiment because of large deviations (Table 2).

The rats were treated with a daily oral administration of AM or AM and SP for 7 d. The plasma levels of PX after the final dosing are shown in Fig. 3. In the multiple dose groups of AM and SP, the plasma levels were also higher than those after multiple doses of AM alone from 8 h after the dosing,

Table 3. Pharmacokinetic Parameters of PX and OH-PX after Multiple Oral Administration of AM Alone and in Combination with SP

Parameter	AM alone	AM+SP	AM alone	AM+SP
	PX		OH-PX	
AUC ($\mu\text{g} \cdot \text{h}/\text{ml}$)	193.46 ± 6.12	296.68 ± 76.88	15.82 ± 2.00	14.27 ± 3.01
MRT (h)	8.26 ± 0.63	9.47 ± 1.03	11.55 ± 1.82	14.76 ± 5.34
k_e (h^{-1})	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.02	0.07 ± 0.02
$t_{1/2}$ (h)	5.72 ± 0.44	6.66 ± 0.77	8.00 ± 1.26	10.23 ± 3.70
$C_{\max}$ ($\mu\text{g}/\text{ml}$)	18.26 ± 1.23	20.21 ± 6.03	1.29 ± 0.27	0.72 ± 0.18
Bioavailability (%)	83.2 ± 2.6	128.0 ± 33.0	—	—
$AUC_{\text{OH-PX}}/AUC_{\text{PX}}$	—	—	0.08 ± 0.01	0.05 ± 0.01^a

Dose: AM, 7.5 mg PX equivalent/kg; SP, 120 mg/kg. Each value represents the mean $\pm$ S.D. ($n=4-5$). $a) p<0.05$ compared with AM alone.

Fig. 3. Plasma Concentration–Time Profiles of PX (A) and OH-PX (B) after Multiple Oral Administration of AM Alone and in Combination with SP

Each point represents the mean $\pm$ S.D. ($n=4-5$). Dose: AM, 7.5 mg PX equivalent/kg; SP, 120 mg/kg. $\circ$, PX and OH-PX after AM alone; $\bullet$, PX and OH-PX after AM+SP. $a) p<0.05$ compared with AM alone.

with 1.5 times larger AUC than that after AM alone. Consequently, the bioavailability of the coadministration group was 1.5 times as large as that of the single dose group (Table 3).

Little is known of the hydroxylation of PX or the disposition of the metabolite in rat. OH-PX in plasma was determined after the oral administration of AM or AM and SP. The results are shown in Figs. 2 and 3. The plasma concentrations of OH-PX were extremely low in rats, and the levels until 10 h after dosing were below one tenth of those of PX. The coadministration with SP, either by simultaneous dosing or preloading, did not appear to affect the plasma concentration profiles of OH-PX. However, the AUC ratio ($AUC_{\text{OH-PX}}/AUC_{\text{PX}}$) for the coadministration and preloading groups was 5.8 and 5.5%, respectively, whereas that for AM alone was 10.7%. Thus, the hydroxylation of PX was found to significantly decrease by preloading with SP ($p<0.05$). In multiple dosing, the AUC ratio was also significantly decreased by the simultaneous administration of SP ($p<0.05$).

Intestinal and Hepatic Drug Metabolizing Enzyme Activities In order to clarify whether the enzyme activities are inhibited by SP, a potent inhibitor, hepatic and intestinal metabolizing enzyme activities were determined after single and multiple oral administrations of AM, SP or the two in combination, with the intention of determining the elimination of PX. Aryl esterase activity in the intestinal mucosa and liver,

which is related to the metabolic conversion of AM to PX, and total CYP content, which plays the major role in PX hydroxylation, were hardly influenced by dosing with AM or SP or the coadministration of AM and SP, even after multiple administrations. When the UDPGT activity of the liver was assayed after a single oral administration of AM or AM and SP, the activity was also not affected by the treatment. From this result, the assay of UDPGT after the multiple dosing was omitted.

The negligible effect of these treatments on the intestinal and hepatic drug metabolizing enzyme activities supported the data that the elimination of PX from the circulation was not significantly influenced by the administration of AM, or by AM in combination with SP, although a possibility that CYP isozymes might partly be influenced by the coadministration with SP cannot be completely ruled out.

Ulcerogenic Activity The ulcerogenic effect of PX on the stomach of rat was about 1/3 that of indomethacin (IND) and 5 times greater than that of phenylbutazone.²³⁾ Ulcerogenic activities were thus tested after repeated dosing of these drugs for 7 d. Only a few ulcers (2–3 in the duodenum) were observed in the gastrointestinal mucosa in SP and AM+SP groups. However, gastrointestinal damage was not detected in rats which received AM alone, indicating that the ulcerogenic activity of AM was much less than that of in-

domethacin reported by us.²⁴⁾

DISCUSSION

The use of multiple drug therapy in the treatment of many diseases has led to problems with drug interactions. Many drug interactions are mainly the result of the interference of one drug with the metabolism of another and a subsequent increase or decrease in the clearance of the latter drug.²⁵⁾ The present study was designed to evaluate the effect of SP on the pharmacokinetics of AM and PX, and to clarify the possible mechanism for a pharmacokinetic drug interaction between both drugs.

Drug interactions are an important factor in the etiology of adverse reactions. Numerous drug interactions caused by cimetidine, a potent H_2 -receptor antagonist, have been documented in human.^{26–31)} Cimetidine also decreases the elimination of PX in rat and man.⁶⁾ Our study in rats has demonstrated that a single oral coadministration of AM and SP did not significantly affect the elimination of PX converted from AM, although the production of OH-PX was partly decreased by this coadministration ($p < 0.05$). When SP was preloaded at 2 h before the dosing of AM, the disposition of PX was delayed and the AUC was 1.8 times larger than that after AM alone, but not significant. These indicate that the coadministration of AM and SP does not result in a relevant interaction, although repeated administration and preloading with SP might partly elicit a metabolic interaction. The metabolic pathway of AM and PX is shown in Fig. 4.

Compounds such as cimetidine³¹⁾ and macrolide antibiotics,³²⁾ which inhibit drug metabolism *in vivo*, have been demonstrated to act by direct inhibition of CYP-dependent oxidation. PX is metabolized by CYP2C9,⁷⁾ and SP inhibits both CYP2C9 and 2C10.⁸⁾ However, our data on the inhibitory effect of drug metabolizing enzyme activities and on the elimination of PX (Fig. 1) approximately denied a possibility of the inhibition of CYP by SP. Consequently, other mechanisms for the interaction must be considered. Since SP is also metabolized by CYP2C9,⁷⁾ the competition of CYP2C between PX and SP might be involved in the decrease in the elimination and hydroxylation of PX in the preloaded rats. The results that the effect of SP was observed in the preloading group but not in the coadministration group led us to postulate the reason as follows. In the preloading study with SP, SP which was absorbed and reached the liver in a large quantity would occupy the oxidases (CYPs) in the liver, and thus result in a partly decreased metabolism of PX which later reached the organ, consequently giving high plasma levels of PX and relatively low levels of OH-PX. In coadministration rats, PX converted in the gastrointestinal tract was rapidly absorbed from the intestine and passed the liver more quickly than SP, this being indicated by a comparatively shorter t_{max} (3–5 h) after oral dosing. It is reported that PX was well absorbed from the stomach and upper small intestine, and that AM was mainly from the duodenum,³³⁾ while SP is a long acting sulfa drug due to being very sparingly soluble in water. Additionally, SP may inhibit the metabolism of PX by an unknown mechanism, as reported with IND.³⁴⁾

Gastrointestinal bleeding caused by NSAIDs is the most dangerous when it results from a mixed pharmacokinetic/pharmacodynamic interaction. Since IND causes great gas-

Fig. 4. Metabolic Pathway of AM and PX

trointestinal damage, and the intestinal absorption was significantly decreased,³⁵⁾ the gastrointestinal side effect of AM and AM+SP was estimated. However, an interaction involving the drug absorption process is unlikely, since the gastrointestinal damage was scarcely detected in rats which received AM and SP. In addition, this was also supported by the data that the coadministration of AM and SP did not decrease the C_{max} or plasma concentrations of PX compared with those after AM alone (Fig. 2).

PX is extensively metabolized by hepatic CYP enzymes, principally to the hydroxyl metabolite in man.⁷⁾ In rats, an average of 25.2% of the dose was recovered in urine as OH-PX, with approximately two-thirds (17.2%) in the form of glucuronide conjugates.³⁶⁾ In this study, OH-PX in plasma was determined after the oral administration of AM and AM+SP (Fig. 3). The plasma concentrations of OH-PX were very low compared with those of PX after both administrations. The AUC_{OH-PX}/AUC_{PX} ratio was decreased to some extent in the repeatedly coadministered and preloaded groups, compared with that of AM alone. This indicates that the metabolism of PX was partly inhibited by SP coadministered or preloaded, probably due to the competition of CYP enzymes between PX and SP. However, the metabolism of PX in rat was much lower than that predicted from the human data reported (75% of a dose is excreted as either OH-PX or its glucuronide³⁷⁾), although the glucuronide in the plasma was not determined. This would mainly be due to the low percent of unbound fraction (2.6%), which can be metabolized by CYP enzymes. Consequently, the minor drug interaction between PX and SP observed in this study was partly ascribed to the low extent of PX metabolism in the liver. The UDPGT activity in the

liver was also not inhibited by the coadministration of SP, indicating that the glucuronidation of OH-PX would not be affected by SP dosed concomitantly.

It has been questioned whether the hydrolysis of the ethoxycarbonyl side chain of AM occurs in the intestinal tract or mucosa. Our data on oral dosing indicated the rapid hydrolysis of the ester bond. Most of the hydrolysis would probably be catalyzed in the intestinal wall and partly by the luminal enzymes before absorption.

These results obtained by us were contrary to our expectation, such as the inhibition of PX metabolism, delayed elimination and consequent conferring of a dramatic drug interaction. The lower interaction is explained as follows. First, the difference in the absorption rate between PX and SP and in the distribution to the liver, as mentioned above, may weaken the inhibitory effect of SP on PX metabolism. Second, it may be that the CYP2C isoform of rat might partly be functionally different from that of human, although it is reported that the sequence determination for nucleic acids of CYP2C in human shows a 79% similarity to the isoform of rat.³⁸⁾ CYP2C9 has not been detected in rat liver. Third, the low extent of PX metabolism in liver results in the lesser drug interaction between PX and SP.

Small animals metabolize drugs at a more rapid rate than man, and the rate of elimination of drugs from the body is largely dependent on the metabolism of the drugs. Therefore, the extent of pharmacokinetic drug interaction in man may be larger than that in rat, so the possibility of the drug interaction between AM and SP cannot be completely ruled out in human.

In conclusion, a pharmacokinetic drug interaction between AM and SP was hardly observed by concurrent administration of the two drugs, although there were some increases in plasma concentrations of PX after preloading and multiple dosing with SP. Coadministration of SP slightly but significantly decreased the metabolism of PX to OH-PX and the AUC_{OH-PX}/AUC_{PX} ratio. However, the extent of PX converted to OH-PX in plasma was comparatively low (below 10%). Significant inhibition of the metabolic enzyme activities by SP, which is a potent inhibitor of CYP2C9, was not observed in rats.

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