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Predictability of metabolism of ibuprofen and naproxen using chimeric mice with human hepatocytes

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Running title: Metabolism of ibuprofen using human liver chimeric mice

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Abbreviations

P450, cytochrome P450; NSAIDs, non-steroidal anti-inflammatory drugs; UGT, UDP-glucuronosyltransferase; SULT, sulfotransferase; h-PXB mice, human chimeric mice; r-PXB mice, rat chimeric mice; PK, pharmacokinetics; LC/MS/MS, liquid chromatography/tandem mass spectrometry; SCID, severe combined immunodeficiency disease; RI, replacement index

Abstract

Prediction of human drug metabolism is important for drug development. Recently, the number of new drug candidates metabolized by not only cytochrome P450 (P450) but also non-P450 has been increasing. It is necessary to consider species differences in drug metabolism between humans and experimental animals. We examined species differences of drug metabolism, especially between humans and rats, for ibuprofen and (*S*)-naproxen as non-steroidal anti-inflammatory drugs (NSAIDs), which are metabolized by P450 and UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), and amino acid *N*-acyltransferase for taurine conjugation in liver, using human chimeric mice (h-PXB mice) repopulated with human hepatocytes and rat chimeric mice (r-PXB mice) transplanted with rat hepatocytes. We performed the direct comparison of excretory metabolites in urine between h-PXB mice and reported data for humans as well as between r-PXB mice and rats after administration of ibuprofen and (*S*)-naproxen. Good agreement for urinary metabolites (% of dose) was observed not only between humans and h-PXB mice but also between rats and r-PXB mice. Therefore, the metabolic profiles in humans and rats reflected those in h-PXB mice and r-PXB mice. Our results indicated that h-PXB mice should be helpful for predicting the quantitative metabolic profiles of drugs mediated by P450 and non-P450 in liver, and r-PXB mice should be helpful for evaluation of species differences in these metabolic enzymes.

Introduction

It is important to predict human drug metabolism and pharmacokinetics (PK) during the pre-clinical stage in the pharmaceutical industry because PK contributes to efficacy and toxicity. Recently, the attrition rate during drug development has been decreasing as a result of improvement of predictability with regard to human metabolism (Kola and Landis, 2004). The number of new drug candidates metabolized by not only cytochrome P450 (P450) but also non-P450 has been increasing, and they show diverse chemical structures, including a carboxyl group to avoid metabolism by P450. Various approaches to predict human metabolism and PK using an in vitro metabolic system with human liver microsomes, S9 fraction, and hepatocytes have been reported (Obach *et al*., 1997; Nagilla *et al*., 2006; Brown *et al*., 2007; Fagerholm, 2007; Stringer *et al*., 2008; Anderson *et al*., 2009; Chiba *et al*., 2009; Hallifax *et al*., 2010; Dalvie *et al*., 2009). However, these methods have some limits for prediction. The above reports described that it was difficult to predict secondary metabolism owing to the complication of multiple drug metabolic enzymes such as P450 and non-P450 because the success rate of corresponding to the observed metabolites using hepatocytes was low (Anderson *et al*., 2009; Dalvie *et al*., 2009).

Chimeric mice with humanized liver, generated using urokinase-type plasminogen activator ($uPA^{+/+}$)/severe combined immunodeficiency (SCID) mice repopulated with human hepatocytes (h-PXB mice; Phoenixbio, Co., Ltd., Hiroshima, Japan) have been reported

(Tateno *et al*., 2004). These mice are transplanted with about 80% of human hepatocytes, and the expression levels and activities of P450 and non-P450 in the liver of h-PXB mice are similar to those of humans (Katoh *et al*., 2004, 2005; Nishimura *et al*., 2005; Katoh and Yokoi, 2007; Kitamura *et al*., 2008).

Some specific metabolites were qualitatively detected in the urine and plasma of h-PXB mice (Inoue *et al*., 2009; Yamazaki *et al*., 2010; Serres *et al*., 2011; Sanoh *et al*., 2012a). Thus, h-PXB mice could be a good in vivo model for predicting drug metabolism in humans.

However, previous investigations for quantitative prediction as well as qualitative prediction of human metabolites involved in multiple metabolic pathways from data in h-PXB mice have been insufficient.

Racemic ibuprofen and (*S*)-naproxen have been widely used as non-steroidal anti-inflammatory drugs (NSAIDs), which are metabolized by certain metabolic enzymes such as P450 and UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), and amino acid *N*-acyltransferase for taurine conjugation in liver (Fig.1, 2). These metabolites are mainly excreted in urine. Furthermore, species differences in the metabolism of ibuprofen and (*S*)-naproxen have also been reported between rats and humans (Mills *et al*., 1973; Sugawara *et al*., 1978).

In this study, rat-chimeric mice (r-PXB mice) containing rat hepatocytes were used to compare the metabolism and PK between rats and humans, as well as h-PXB mice, as an in

vivo approach (Tateno *et al*., 2004; Emoto *et al*., 2005; Yamazaki *et al*., 2010; Sanoh *et al*., 2012a). The aim of this study was to assess the quantitative predictability of the metabolism by P450 and non-P450 by examining urinary excreted metabolites in h-PXB mice and r-PXB mice after administration of ibuprofen and (*S*)-naproxen.

Materials and Methods

Chemicals

2-(4-Isobutylphenyl)-propionic acid (Ibuprofen) and 2-(3-benzoylphenyl)-propionic acid (ketoprofen) were purchased from Wako Pure Chemicals (Osaka, Japan). (*S*)-(+)-2-(6-Methoxy-2-naphthyl) proprionic acid ((*S*)-Naproxen) was purchased from Cayman Chemical (Ann Arbor, Michigan). 2-[4-(2-carboxypropyl)phenyl]-propionic acid (Carboxy ibuprofen), 2-[4-(2-hydroxy-2-methylpropyl) phenyl]-propionic acid (2-hydroxylibuprofen), (*S*)-(+)-2-(6-Hydroxy-2-naphthyl)-proprionic acid ((*S*)-*O*-desmethylnaproxen), and (*S*)-naproxen acyl-beta-D-glucuronide were obtained from Toronto Research Chemicals Inc. (North York, ON, Canada). Ibuprofen taurine conjugate was synthesized in accordance with Shirley *et al*. (1994). All of the other reagents and solvents were commercial products of the highest available grade or analytical grade.

Animals

The present study was approved by the animal ethics committee and was conducted in accordance with the regulations on the use of living modified organisms of Hiroshima University. Sprague-Dawley (SD) rats (6 weeks of age) and severe combined immunodeficiency disease (SCID mice) (10 weeks of age) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). h-PXB mice and r-PXB mice (10 weeks of

age), transplanted with human and rat hepatocytes, respectively, were prepared by PhoenixBio Co. Ltd. (Hiroshima, Japan). All animals were housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle with free access to tap water and food.

Human hepatocytes of a donor (African-American boy, 5 years old) were obtained from BD Biosciences (San Jose, CA). Rat hepatocytes for the preparation of r-PXB mice were isolated from the liver of SD rats (4 weeks of age, male). The replacement ratio of host hepatocytes with human or rat hepatocytes, calculated as the replacement index (RI), was determined by measurement of the level of human or rat albumin in blood collected from the tail vein of each PXB mouse (Tateno *et al.*, 2004; Emoto *et al*., 2005). Average RI values of h-PXB mice and r-PXB mice used in this study were 78% and nearly 100%, respectively.

Administration of ibuprofen and (*S*)-naproxen

Ibuprofen and (*S*)-naproxen solution (5mL/kg) were administered orally to each animal at 20mg/kg and 10mg/kg body weight, respectively, which included 0.5% carboxy methyl cellulose with requisite minimum amount of potassium hydroxide for solution. After treatment of ibuprofen and (*S*)-naproxen, pooled urine samples were collected until 24 and 48h, respectively.

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Analysis and quantitation of ibuprofen, (*S*)-naproxen, and their metabolites

Ibuprofen

Pooled urine (20μL) was mixed with 0.1% formic acid (500μL) and internal standard solution (ketoprofen 30μg/mL, 10μL). These mixtures were absorbed to the MonoSpin® C18 (GL Sciences Inc., Tokyo, Japan) for solid phase extraction. Samples purified by elution with 50% acetonitrile were subjected to liquid chromatography/tandem mass spectrometry (LC/MS/MS). The concentrations of ibuprofen acyl glucuronide, carboxy ibuprofen acyl glucuronide and 2-hydroxyibuprofen acyl glucuronide were determined as increased amounts of ibuprofen, carboxy ibuprofen and 2-hydroxyibuprofen by hydrolysis using 1M sodium hydroxide before solid phase extraction.

(*S*)-Naproxen

Pooled urine (20μL) was mixed with acetonitrile (30μL). After centrifugation, the supernatants with 10mM ammonium acetate were subjected to LC/MS/MS.

The concentrations of (*S*)-6-*O*-desmethyl naproxen glucuronide were determined as increased amounts of 6-*O*-desmethyl naproxen by incubation for 2h at 37ºC using beta-glucuronidase (20μL) in 1M acetate buffer (100μL) after thawing the hepatocytes. The concentration of 6-*O*-desmethyl naproxen sulfate was estimated by subtracting the concentration of 6-*O*-desmethyl naproxen glucuronide from that of total hydrolyzed 6-*O*-desmethyl naproxen

after enzyme deconjugation for 2h at 37ºC using beta-glucuronidase/arylsulfatase (20μL) in 1M acetate buffer. Incubation mixtures were extracted with ethyl acetate (5mL) and internal standard solution (ketoprofen). The organic layer (4mL) was evaporated to dryness, and the residues were dissolved in aqueous acetonitrile (100μL). Aliquots of 10μL were applied to LC/MS/MS.

LC/MS/MS condition

Aliquots (10μL) of urine samples were introduced into the LC system (Agilent Technologies, Santa Clara, CA). The mobile phase condition for ibuprofen and (*S*)-naproxen consisted of 10mM ammonium acetate (A) and acetonitrile (B) through an Inersil ODS-3 column (5μL, 50 x 2.1mm; GL Sciences Inc., Tokyo, Japan) at 40 ºC. The flow rate was set at 0.2mL/min. The starting condition for LC gradient was 90:10 (A/B). From 0 to 5 min, the mobile phase composition was changed to 10:90 (A/B), and this was maintained until 8 min. The gradient was then returned to 90:10 (A/B) linearly from 8 to 8.1 min, and the column was re-equilibrated to the initial condition from 8.1 to 15 min. The elution times of ibuprofen, ibuprofen taurine conjugate, carboxy ibuprofen, 2-hydroxyibuprofen, (*S*)-naproxen, naproxen acyl glucuronide, (*S*)-6-*O*-desmethylnaproxen, and ketoprofen as internal standard were 5.9 min, 5.8 min, 0.9 min, 4.2 min, 5.0 min, 4.9 min, 4.0 min, and 5.0 min, respectively.

The MS/MS experiments were conducted by using API2000 LC/MS/MS systems (Applied

Biosystems, Foster, CA). Mass numbers of the ionization mode, molecular ion, and product ion for the ibuprofen, (*S*)-naproxen, and their metabolites were as follows: ibuprofen m/z = 204.9 $[M-H]^-$ to 158.5, ibuprofen taurine conjugate m/z = 311.9 $[M-H]^-$ to 123.4, carboxy ibuprofen m/z = 235.1 $[M-H]^-$ to 72.6, 2-hydroxy ibuprofen m/z = 221.3 $[M-H]^-$ to 176.9, (*S*)-naproxen m/z = 228.7 $[M-H]^-$ to 168.5, naproxen acyl glucuronide m/z = 404.8 $[M-H]^-$ to 169.1, (*S*)-6-*O*-desmethylnaproxen m/z = 214.7 $[M-H]^-$ to 170.4, and ketoprofen m/z = 253.2 $[M-H]^-$ to 208.7.

Results

Predictability of metabolic profiles of ibuprofen in humans

Proposed metabolic pathways of ibuprofen were previously reported from the urinary metabolic profile excreted in humans after oral administration of ibuprofen (Fig.1). Six metabolites, ibuprofen acyl glucuronide, ibuprofen taurine conjugate, carboxy ibuprofen, carboxy ibuprofen glucuronide, 2-hydroxy ibuprofen, and 2-hydroxy ibuprofen acyl glucuronide were predominantly detected in urine (Shirley *et al*., 1994; Kepp *et al*., 1997). The percentage values in Table 1 indicate urinary excreted metabolites in relation to the dose (% of dose) after oral administration of ibuprofen in humans (400mg and 600mg/person), h-PXB mice (20mg/kg), rats (20mg/kg), and r-PXB mice (20mg/kg). These metabolites observed in humans were also identified in h-PXB mice, rats, and r-PXB mice. Excreted unchanged form of ibuprofen in urine was negligible in all animals in this study (below 2% of the dose). Excreted acyl glucuronide conjugates in human urine were higher than those of rats, whereas amount of 2-hydroxy ibuprofen in human was lower than that of rats. We directly compared six urinary metabolites (% of dose) between humans and rats (Fig.3A). There were weak correlations (r^2= 0.471, p= 0.132). The correlations reflect species differences in the excretory metabolic profile between humans and rats. To investigate whether these differences reflect each chimeric mice, we directly compared the excreted metabolites between humans and h-PXB mice. This result showed good correlation (r^2= 0.863, p= 0.007) (Fig.3B).

Additionally, a good correlation was also found between rats and r-PXB mice (r^2= 0.928, *p*= 0.002) (Fig.3C), whereas the relationship between h-PXB mice and r-PXB mice was weaker (r^2= 0.286, *p*= 0.274) (Fig.3D). These data suggested that the excretory metabolic profiles in humans and rats qualitatively reflected those of h-PXB mice and r-PXB mice, respectively. In case of comparison with SCID mice, host of chimeric mice, a low correlation was observed between humans and SCID mice (r^2= 0.246, *p*= 0.317) as well as h-PXB mice and SCID mice (r^2= 0.129, *p*= 0.484) (Fig.3E, F).

Predictability of metabolic profiles of (*S*)-naproxen in humans

(*S*)-Naproxen is metabolized into four metabolites: (*S*)-naproxen acyl glucuronide, (*S*)-6-*O*-desmethylnaproxen, and the latter's metabolites, (S)-6-*O*-desmethylnaproxen sulfate and (S)-6-*O*-desmethylnaproxen acyl glucuronide, which were reported to be mainly excreted in the urine of humans (Fig.2) (Sugawara *et al*., 1978). (*S*)-Naproxen was also excreted at negligible levels. Table 2 shows the percentage of each urinary metabolite after oral administration of (*S*)-naproxen in humans (200mg/person), h-PXB mice (10mg/kg), rats (10mg/kg), and r-PXB mice (10mg/kg). Four metabolites reported in humans were also found in the urine of h-PXB mice, rats, and r-PXB mice. Excreted naproxen acyl glucuronide and 6-*O*-desmethylnaproxen acyl glucuronide in human urine were higher than those of rats, whereas 6-*O*-desmethylnaproxen and its sulfate in human were lower than that of rats.

We compared the % dose of these excretory metabolites with those of chimeric mice. Amount of naproxen acyl glucuronide, which was mainly observed in human urine, and that of 6-desmethylnaproxen, which was a low metabolite, corresponded to those of h-PXB mice. On the other hand, the amount of 6-*O*-desmethylnaproxen sulfate, which was mainly observed in rats, and that of naproxen acyl glucuronide, which was lower, were in close agreement with those of r-PXB mice. Differences of excretory metabolic profiles between humans and rats were similar to those between h-PXB mice and r-PXB mice.

Discussion

Identification of primary metabolites contributes to drug design for stable metabolic analogues. Not only primary metabolites but also secondary metabolites could be involved in efficacy and toxicity via biotransformation.

It is also necessary to reflect on species differences of isoform composition, expression, and activity of drug metabolic enzymes between humans and experimental animals (Martignoni *et al*., 2006). We considered that h-PXB mice with high replacement of human hepatocytes may be useful for prediction of human metabolism because the expression levels and activities of both P450 and non-P450 enzymes reflect those of the donor hepatocytes (Yoshitsugu *et al*., 2006; Yamasaki *et al*., 2010). Sanoh *et al.* (2012b) demonstrated the predictability of human PK of 13 model compounds, including ibuprofen and (*S*)-naproxen, metabolized by P450 and non-P450, using h-PXB mice. In case of ibuprofen, the predictaility of in vivo intrinsic clearance in h-PXB mice reflected that of observed human (Sanoh *et al*., 2012b).

Ibuprofen was metabolized by CYP2C9 and UGT2B7 (Hamman *et al*., 1997; Buchheit *et al*., 2011). Additionally, taurine conjugate of ibuprofen was identified in the urine of humans as a minor metabolite (Shirley *et al*., 1994). (*S*)-Naproxen was metabolized by CYP2C9, CYP1A2, UGT2B7, and SULT1A1 (Rodrigues *et al*., 1996; Bowalgaha *et al*., 2005; Falany *et al*., 2005).

CYP2C9 is one of the most abundant P450 enzymes in human liver. CYP2C9 metabolizes

approximately 20% of clinical drugs, including a number of drugs with narrow therapeutic ranges. UGT2B7 also contributes to the metabolism of numerous clinical drugs (Williams *et al.*, 2004).

Ibuprofen and (*S*)-naproxen are suitable as representative model compounds to elucidate the predictability of multiple metabolic pathways associated with P450 and non-P450 using h-PXB mice. Metabolites of ibuprofen and (*S*)-naproxen were reported to be excreted in urine, which suggested that the kidneys are the main excretion route (Shirley *et al*., 1994; Kepp *et al*., 1997, Sugawara *et al*., 1978). Furthermore, we used r-PXB mice as a control model in consideration of species differences between humans and rats in this study.

Six metabolites of ibuprofen, which were identified in humans, were also found in the urine of h-PXB mice, r-PXB mice, and rats. On the other hand, fecal excretion of these metabolites was low (data not shown). These findings suggested that h-PXB mice reflected species differences of the main excretory pathways of cefmetazole (Okumura *et al.*, 2007). We could confirm species differences in the amounts of urinary excretion of these metabolites between humans and rats because a weak correlation (r^2= 0.471, p= 0.132) was observed, as shown in Fig.3A. There were good correlations between humans and h-PXB mice (r^2= 0.863, p= 0.007), as well as rats and r-PXB mice (r^2= 0.928, p= 0.002) (Fig3B, C). Therefore, species differences of urinary excretion of metabolites between humans and rats reflect the relationship between h-PXB mice and r-PXB mice.

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(*S*)-Naproxen is metabolized in humans by acyl glucuronidation, *O*-demethylation, and further sulfation and glucuronidation. Four metabolites in urine after administration were found in h-PXB mice, r-PXB mice, and rats. Species differences of excretory metabolites between humans and rats reflect the levels in humans and rats because amounts of each urinary metabolite were similar between humans and h-PXB mice as well as rats and r-PXB mice, in common with the case for ibuprofen.

We used h-PXB mice for which the average RI values were about 80%. The contribution of the remaining 20% of host hepatocytes may have influenced the predictability. Direct comparison of excretory metabolites between humans and SCID mice as host mice gave a value of r^2= 0.246 (p= 0.317) (Fig.3E). In addition, there was no correlation (r^2=0.129, p= 0.484) between h-PXB mice and SCID mice (Fig.3F) although, in vitro intrinsic clearance of ibuprofen in SCID mouse hepatocytes was similar to that of h-PXB mouse hepatocytes (Sanoh *et al*., 2012b). This result suggested that the remaining host mouse hepatocytes did not affect the predictability using h-PXB mice despite species differences between humans and SCID mice being observed. In case of r-PXB mice, it is not necessary to consider the remaining host mouse hepatocytes because RI of rat hepatocytes in liver of r-PXB mice is about 100%.

In this study, analysis of the predictability using h-PXB mice and r-PXB mice was conducted by oral administration. It is also necessary to consider the effects of the intestine, which is not

humanized in h-PXB mice, in cases of oral administration. We checked comparison of recovery metabolites in h-PXB mice between after intravenous and oral administration of ibuprofen. Since these results in good correlations (r^2=0.900, p= 0.004), metabolic activities of ibuprofen in mouse intestine may be negligible (data not shown).

Our results using ibuprofen and (*S*)-naproxen indicated that in vivo metabolic activity of P450 and non-P450, such as those involving UGT, SULT, and amino acid *N*-acyltransferase in h-PXB mice and r-PXB mice, should be similar to those of humans and rats at least.

In this study, r-PXB mice were used as the control animal for transplantation of hepatocytes. The predictability using h-PXB mice may improve when the metabolic profiles of r-PXB mice reflect those of rats.

In conclusion, our results suggest that the combined use of h-PXB mice and r-PXB mice may be helpful for quantitative prediction of species differences of drug metabolism during the early stages of drug development in the pharmaceutical industry.

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Author Contributions

Participated in research design: Sanoh, Sugihara, Kotake, Tayama, Horie, Kitamura, and Ohta

Conducted experiments: Sanoh and Horiguchi

Contributed new reagents or analytical tools: Sugihara, Kotake, Uramaru, Ohshita, and Tateno

Performed data analysis: Sanoh and Horiguchi

Wrote or contributed to the writing of the manuscript: Sanoh, Kotake, and Ohta

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Footnote

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Figure titles and legends

Fig.1 Proposed metabolic pathways of ibuprofen in humans. This figure was drawn from the data of Shirley *et al*.(1994), and Kepp *et al*.(1997).

Fig.2 Proposed metabolic pathways of (*S*)-naproxen in humans. This figure was drawn from the data of Sugawara *et al*. (1978).

Fig. 3 Cross-species comparison of ibuprofen metabolites excreted in urine after oral administration of ibuprofen in h-PXB mice, humans, r-PXB mice, rats, and SCID mice. (A) Comparison of each urinary excreted metabolite (% of dose) between humans and rats, (B) Humans vs. h-PXB mice, (C) Rats vs. r-PXB mice, (D) h-PXB mice vs. r-PXB mice, (E) Humans vs. SCID mice, (F) h-PXB mice vs. SCID mice.

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Table 1 Cumulative urinary excretion of six metabolites of ibuprofen.

Species	Urinary excreted metabolites % of dose					
	Ibuprofen acyl glucuronide	Ibuprofen taurine conjugate	Carboxy ibuprofen	Carboxy ibuprofen glucuronide	2-Hydroxy ibuprofen	2-Hydroxy ibuprofen acyl glucuronide
h-PXB mice	11.3±8.5	0.4±0.4	5.4±2.3	7.0±3.4	2.4±2.3	8.4±4.7
Humans	11.6±7.6	1.5±0.5	13.5±3.7	11.6±7.3	5.9±2.7	28.1±8.5
r-PXB mice	0.9±0.7	0.2±0.2	4.4±3.5	1.2±1.1	8.8±6.9	23.8±17.4
Rats	0.2±0.1	$8.0 \times 10^{-3} \pm 2.0 \times 10^{-3}$	2.9±0.5	0.8±0.6	34.5±9.8	19.8±2.4
SCID mice	2.5±1.7	0.5±0.3	5.3±2.5	0.2±4.3	1.5±0.6	5.3±2.8

Human data (mean ± S.D., n=4) after oral administration of ibuprofen (600mg/person) were cited from Kepp *et al*.(1997). Data on amount of ibuprofen taurine conjugate (mean ± S.E., n=4) in humans after oral administration (400mg/person) were cited from Shirley *et al*.(1994). Each value of % metabolites of h-PXB mice, r-PXB mice, rats, and SCID mice after oral administration (20mg/kg) is the mean ± S.D. of n=8, 3, 3, and 3 respectively.

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Table 2 Cumulative urinary excretion of four metabolites of (*S*)-naproxen.

Species	Urinary excreted metabolites % of dose			
	(*S*)-Naproxen acyl glucuronide	(*S*)-6-*O*-Desmethyl naproxen	(*S*)-6-*O*-Desmethyl naproxen acyl glucuronide	(*S*)-6-*O*-Desmethylnaproxen sulfate
h-PXB mice	**26.5±6.6**	**0.2±0.2**	**1.0±0.6**	**12.4±3.1**
Humans	**25.3±6.7**	**0.9±0.2**	**8.3±2.0**	**10.8±0.5**
r-PXB mice	**2.9±2.9**	**2.5±1.4**	**0.5±0.5**	**57.3±6.1**
Rats	**1.2±0.7**	**5.3±3.8**	**1.8±0.4**	**56.9±8.7**

Human data (mean ± S.D., n=3) after oral administration (200mg/person) were cited from Sugawara *et al.*(1978). Amounts of acyl glucuronide form were determined by hydrolysis with beta-glucuronidase. Amount of sulfate of desmethyl naproxen was determined by hydrolysis with 2N HCl (Sugawara *et al.*,1978). Data of % metabolites of h-PXB mice, r-PXB mice, and rats after oral administration (10mg/kg) are mean ± S.D. of n=3.

Fig. 1

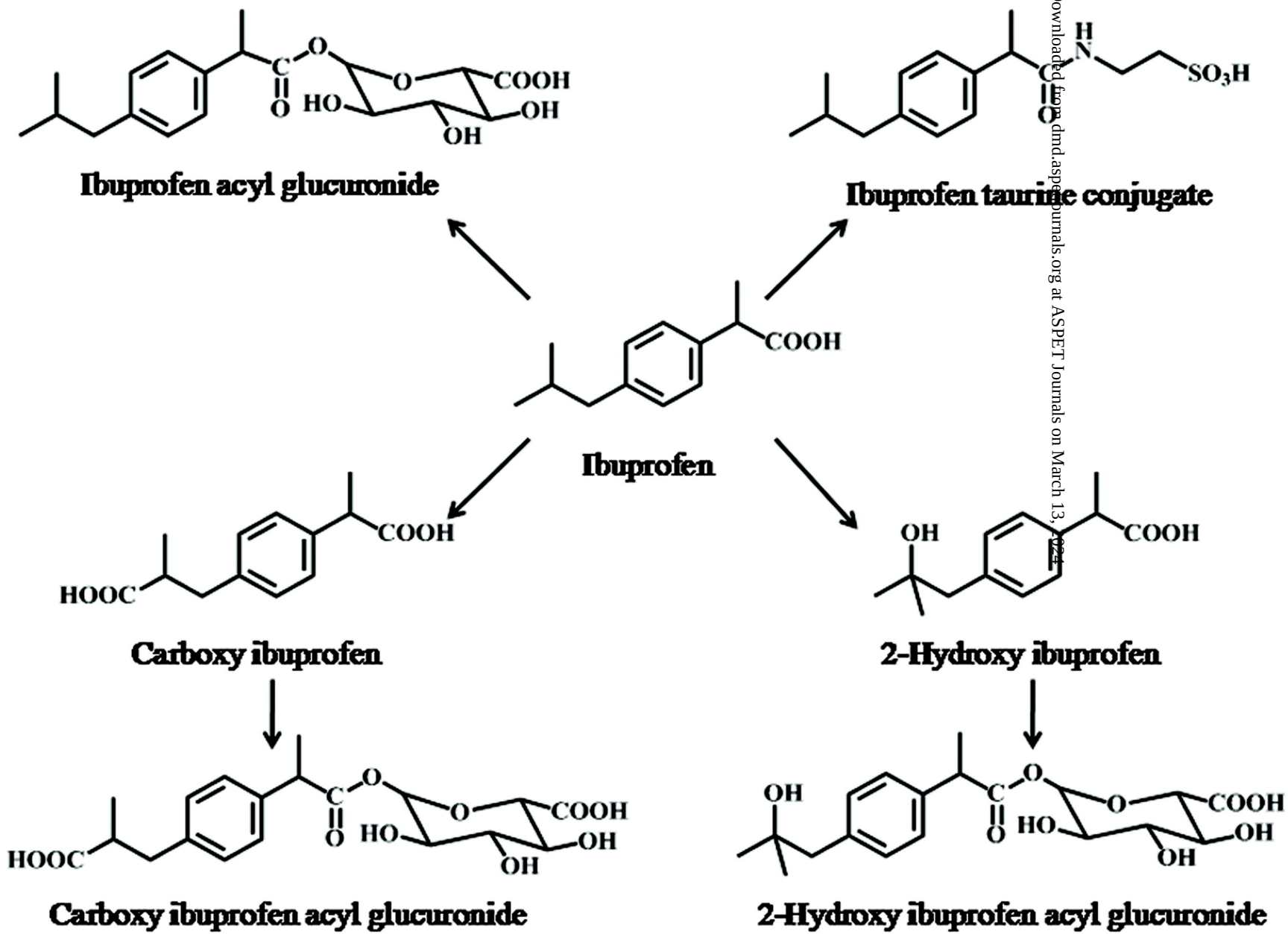

Fig.2

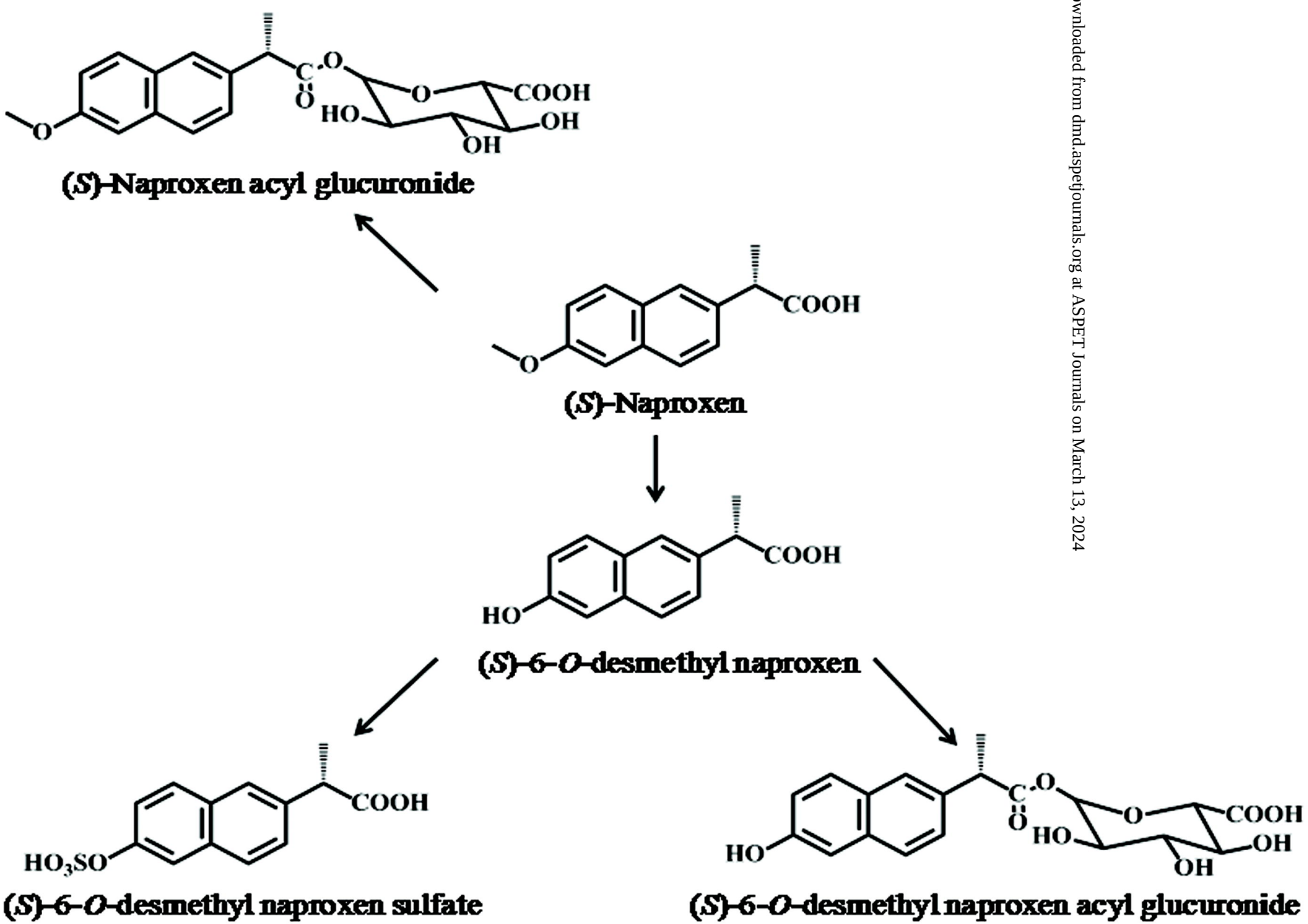

Fig.3

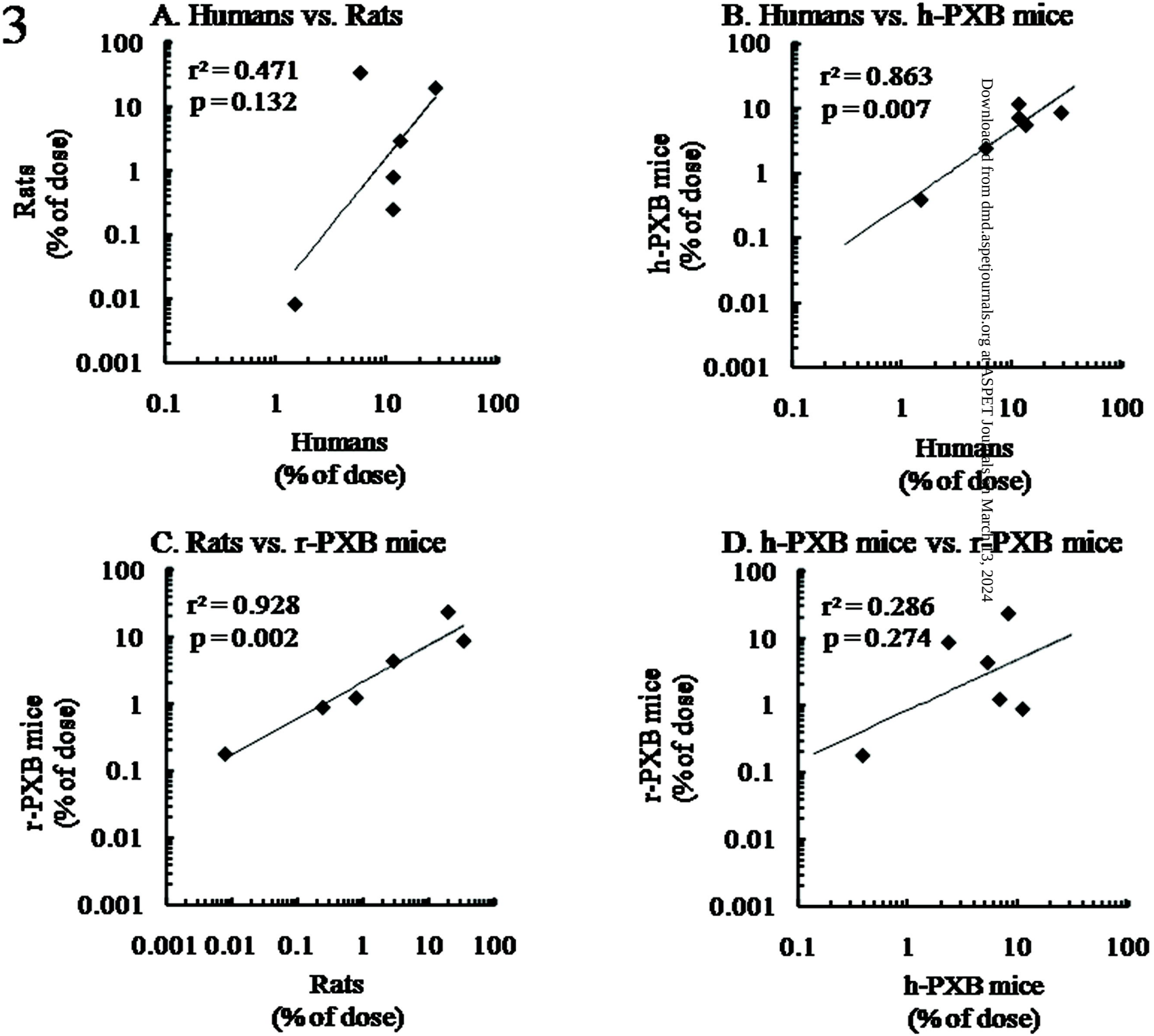

Fig.3