EJP-31844 R2

20100323

Drug interaction between celecoxib and methotrexate in organic anion transporter 3–transfected renal cells and in rats in vivo.

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ABSTRACT

Methotrexate has a clinically important pharmacokinetic interaction with nonsteroidal anti-inflammatory drugs (NSAIDs) mainly through its competition for tubular secretion via the renal organic anion transporter 3 (OAT3). We have previously reported the usefulness of OAT3-transfected renal tubular cells for screening of the drugs which interfere with the pharmacokinetics of methotrexate. Celecoxib, a cyclooxygenase (COX) 2 inhibitor, has not been reported to interact with methotrexate, but the mechanisms are unclear why the interaction is not occurred. The purpose of this study was to evaluate the effect of celecoxib on methotrexate tubular secretion using a renal cell line stably expressing human OAT3 (S2-hOAT3), and to evaluate the pharmacokinetic interaction of the two drugs in rats. [^{3}H]methotrexate uptake into S2-hOAT3 cells was significantly inhibited by celecoxib in a concentration-dependent manner and the Ki value was 35.3 μM. However, methotrexate serum concentrations and urinary excretion of methotrexate over 24 h in rats were not affected by celecoxib (50, 200 mg/kg). Celecoxib serum concentrations were increased by the increase in celecoxib dosage and the maximum drug concentration (Cmax) was 20.6 μM (celecoxib 200 mg/kg), which did not reach the Ki value obtained in the in vitro study. These results indicated that celecoxib inhibited the secretion of methotrexate via hOAT3, which suggested that celecoxib was a substrate of hOAT3. However, co-administration of the two drugs at clinical dosage did not affect the pharmacokinetics of methotrexate, because the serum concentrations did not reach the Ki value. Although the accumulation study using S2-hOAT3 cells was useful to predict the interaction between the new drug and methotrexate in vivo, a comparison of the Ki value with the Cmax in clinical

dosage was necessary to evaluate the degree of this interaction.

INDEX WORDS

Methotrexate, celecoxib, tubular secretion, organic anion transporter, pharmacokinetic interaction.

1. Introduction

Methotrexate and nonsteroidal anti-inflammatory drugs (NSAIDs) are often used concomitantly in clinical practice, such as for the treatment of rheumatoid arthritis and cancer. However, the combination is reported to increase methotrexate-related adverse effects (Daly et al., 1986; Frenia and Long, 1992; Shibayama et al., 2006; Tracy et al., 1992; Tuyss et al., 1986). Therefore, there is need of NSAIDs that do not elevate blood methotrexate levels for use in clinical pain management.

Several mechanisms are known to be involved in the NSAID-induced increases in methotrexate concentrations (Ahern et al., 1988; Brouwers and de Smet, 1994; El-Sheikh et al., 2007; Frenia and Long, 1992; Masada et al., 1997; Murray and Brater, 1993; Nozaki et al., 2007; Tracy et al., 1992; Uwai et al., 2000; Uwai et al., 2004). In our previous study, we found that competition of tubular secretion via organic anion transporter-3 (OAT-3) is the most important mechanism on pharmacokinetic interaction between methotrexate and NSAIDs (Maeda et al., 2008), while others (such as competition for protein-binding sites and inhibition of cyclooxygenase in the kidney) are minor. Our results also showed that drug which did not inhibit the accumulation of methotrexate by the cells, had no potential interactions with methotrexate in the rats, Therefore, OAT3-transfected renal tubular cells are useful for screening of the drugs which interact with the pharmacokinetics of methotrexate. (Maeda et al., 2008).

Celecoxib is a selective cyclooxygenase (COX) 2 inhibitor and it is reported that celecoxib had no significant effect on the pharmacokinetics of methotrexate in patients with rheumatoid arthritis (Karim et al., 1999). Celecoxib has a different chemical structure and higher value of pKa (pKa=11.1) as

compared with other NSAIDs. Therefore, we speculated that celecoxib is not a substrate of OAT, which is the reason why celecoxib had no significant effect on the pharmacokinetics of methotrexate. The purpose of this study was to evaluate the competition between celecoxib and methotrexate by using proximal tubule S2 cells stably expressing hOAT3 (S2-hOAT3 cells) (Takeda et al., 2002) and then to examine the pharmacokinetic interaction between methotrexate and celecoxib using rats.

2. METHODS

2.1 Materials

[3′,5′,7-^{3}H]Methotrexate disodium salt (27. 7 Ci/mmol) and [methoxy-^{14}C]inulin (3.5 mCi/mmol) were purchased from Moravek Biochemicals, Inc. (Brea, CA, USA). Acetaminophen, sulindac and loxoprofen were obtained from Sigma Chemical Co. (Tokyo, Japan). Celecoxib was kindly provided by Pfizer Inc. (Groton, CT, USA). S2-hOAT3 cells, which were established by introducing the human OAT3 gene into SV40-transfected mouse proximal tubule cells, were the generous gift of Dr. Endou (Kyorin University, Tokyo, Japan). This cell line highly and permanently expresses hOAT3 (Takeda et al., 2002).

2.2 In vitro uptake examination

The methods used to examine uptake of methotrexate into S2-hOAT3 cells have been described previously (Maeda et al., 2008). In brief, S2-hOAT3 cells were seeded in 12-well insert plates (3 μm, 8×10^{5} pore/cm^{2}: BD Falcon; Becton Dickinson Co., Lincoln Park, NJ, USA) at a density of 1×10^{5} cells/well for 7 days. Thereafter, the cells were incubated at the basolateral side for 2 h at 37°C in FBS-free medium containing [^{3}H]methotrexate (26 nM), [^{14}C]inulin, and celecoxib, sulindac, loxoprofen, acetaminophen or vehicle. When the uptake was finished, a 150-μl aliquot of the apical side medium was sampled, and its radioactivity was determined in 5 ml of ACS (Amersham Biosciences, Buckinghamshire, UK) by liquid scintillation counting. Samples in which [^{14}C]inulin transited to the apical side (>1% of samples) were excluded. The uptake examination was stopped by cold phosphate-buffered saline (PBS) and the monolayer was rapidly washed 3 times adequately with cold PBS in each

side. The cells in each well were dissolved with 150 μl of 1 N sodium hydroxide for 24 h at room temperature and were diluted 10 times with distilled water. The radioactivity of each sample (150 μl) was measured using the treatment described above. Samples in which [^{14}C]inulin transited to the apical side (>1% of samples) were excluded. The protein content of the solubilized cells was determined using the bicinchoninic acid assay (Pierce, Rockford, IL, USA). We previously determined the accumulation of methotrexate in not-transfected cells (S2-DNA cells) (Maeda et al., 2008). We found that the accumulations of methotrexate in S2-DNA cells were extremely low and negligible as compared to OAT3-transfected cells. Furthermore, the accumulations of methotrexate in S2-DNA were not inhibited by the NSAIDs.

Celecoxib, sulindac, loxoprofen and acetaminophen were dissolved in dimethyl sulfoxide (DMSO). Each drug was diluted with incubation medium. Adjustments were made so that the final concentration in the incubation medium was 0.1%. We preliminary found that low concentration (0.1%) of DMSO used in this study as vehicle did not interfere the transport of methotrexate in this cell.

2.3 In vivo pharmacokinetic examination in rats

Celecoxib, sulindac, and acetaminophen were suspended with tragacanth gum. Vehicle or an NSAID—celecoxib (50, 200 mg/kg), sulindac (50 mg/kg), or acetaminophen (400 mg/kg)—was administered perorally to each fasting male wistar rat, weighing 265-286g (11-12 weeks old). After 30 min, methotrexate (40 mg/kg) (parenteral methotrexate 50 mg; Wyeth K.K., Tokyo, Japan) was administered as a bolus by intraperitoneal injection (He et al., 1991), and water (3% of body weight) was given orally to obtain enough urine. Each rat

was housed individually in a rat metabolic cage during the experiment. In our previous study, the serum concentrations of NSAIDs were reached the Cmax within 30 min after oral administration of NSAID. This study is performed by following the previous study's method (Maeda et al., 2008). We think that the 30 min period is adequate time to observe the interaction with methotrexate because enough celecoxib was exposed in the plasma.

The methods used for blood and urine collection have been described previously (Maeda et al., 2008). The protocol(s) were approved by the institutional ethics committee.

2.4. Measurement of celecoxib

Serum concentrations of celecoxib were determined by HPLC (Paulson et al., 1999). The chromatographic system (Jasco Co., Tokyo, Japan) consisted of an 880-PU intelligent pump equipped with an 880-51 two-line degasser, 851-AS intelligent autosampler, 875-UV intelligent UV detector, and CO-965 column oven. The column was a Mightysil RP-18 (4.6 × 150 mm, 5 µm; KANTO Chemical Co., Inc., Tokyo, Japan) fitted with an RP-18 guard column (4.6 × 5 mm, 5 µm; KANTO Chemical) at 40°C. The mobile-phase flow rate was maintained at 1.0 ml/min for all drugs.

The mobile phase was a mixture of acetonitrile and phosphate buffer (pH 8.0), 5:5 (v/v). The UV wavelength was set at 250 nm. A serum volume of 50 µl was deproteinized by adding 150 µl of acetonitrile and vortexing for 30 seconds. After centrifugation at 12,000×g for 10 min at 4°C, 20 µl of the supernatant was injected onto the HPLC.

2.5 Statistical analysis

Data were analyzed statistically by one-way analysis of variance followed by Scheffe’s post hoc test (vs. vehicle). P values less than 0.05 were regarded as significant.

3. RESULTS

3.1 Inhibition of methotrexate accumulation in S2-hOAT3 cells by celecoxib

We examined the inhibitory effects of celecoxib on methotrexate accumulation in S2-hOAT3 cells. As shown in Fig. 1, celecoxib inhibited the methotrexate accumulation mediated by hOAT3 in a concentration-dependent manner and the Ki value calculated from linear regression analysis with reciprocal of accumulation percent plot was 35.3 μM. We also confirmed our previous results in this cell line that 100 μM of sulindac and 100 μM of loxoprofen significantly inhibited the uptake of methotrexate, while acetaminophen did not. The effects of sulindac, loxoprofen and acetaminophen in this study were comparable with previous study (Maeda et al., 2008).

3.2 Interaction between methotrexate and celecoxib in rats

As shown in Fig. 2, the serum methotrexate concentrations were not significantly changed by celecoxib (50 mg/kg, $P = 0.1537$; 200 mg/kg, $P = 0.2831$) or acetaminophen (400 mg/kg; $P = 0.9940$). However, they were significantly increased by sulindac (50 mg/kg; $P = 0.0028$). We determined the serum celecoxib concentrations in rats administered celecoxib (50, 200 mg/kg) (Fig.3). The maximum drug concentration (Cmax) was 20.6 μM in the animals administered 200 mg/kg celecoxib.

The percentage of methotrexate excreted in urine over 24 h was not affected by celecoxib and acetaminophen, but the urinary excretion of methotrexate was significantly increased over the period of 2-24 h ($P < 0.05$) by sulindac (Fig. 4). The effects of sulindac and acetaminophen in this study were

comparable with our previous study (Maeda et al., 2008)

4. DISCUSSION

In our previous study, we established an in vitro screening system using S2-hOAT3 cells to evaluate the safety of NSAIDs coadministered with methotrexate. Our previous study suggested that a drug (e.g. acetaminophen) which did not inhibit methotrexate accumulation in vitro did not compete with the tubular excretion of methotrexate and did not affect the methotrexate pharmacokinetics(Maeda et al., 2008).

In the present study, we determined the inhibitory effects of celecoxib and other NSAIDs using S2-hOAT3 cells, and we examined the pharmacokinetic interactions between celecoxib and methotrexate in rats at the concentration of methotrexate that is used during high-dose therapy (He et al., 1991). As shown in Figs. 1 and 2, sulindac, which inhibited the accumulation of methotrexate in S2-hOAT3 cells, increased the serum methotrexate concentrations, while acetaminophen, which exhibited no interaction in the in vitro study, also had no effect in vivo. These results were similar to those in our previous study.

It has been speculated that celecoxib, a selective COX-2 inhibitor, is not a substrate of OAT because it has a different structure and different physical and chemical properties as compared to other NSAIDs (Penning et al., 1997). In the present study, however, we found that celecoxib inhibited methotrexate accumulation in vitro mediated by hOAT3 in a concentration-dependent manner. These results suggested that tubular secretion of methotrexate via hOAT3 was inhibited by celecoxib. On the other hand, we found that serum methotrexate concentrations and urinary excretion of methotrexate over 24 h were not affected by celecoxib in this study. We also measured the celecoxib concentrations in rats following administration of 50 or 200 mg/kg of the drug (Fig. 3). It was revealed

that the celecoxib concentrations were below the Ki value obtained by in vitro study. These results indicated that celecoxib inhibited the methotrexate tubular secretion via hOAT3 but pharmacokinetic interaction is not observed at present dose because serum concentration of celecoxib is not reached to Ki value. Therefore, even if we observed inhibition of methotrexate accumulation in S2-OAT3 cell in vitro, drug interaction at renal tubular secretion in vivo will not be observed if serum drug concentration is not approaching the Ki value. If the dose of celecoxib was increased and its concentrations were higher than Ki value, the pharmacokinetic interaction with methotrexate might have appeared. Although inhibition of methotrexate accumulation in S2-hOAT3 is useful to evaluate possible pharmacokinetic interaction with methotrexate in vivo, this analysis becomes less useful if such inhibitory levels in vivo cannot be achieved.

It has been reported that total plasma celecoxib concentrations were below 1.42 μM in human volunteers who received a single application of 200 mg celecoxib (Jayasagar et al., 2002) and were 10.5 μM in those who received 600 mg BID for 7 days (Paulson et al., 1999). Considering Cmax obtained in this study and these literatures, the dose of 50 mg/kg celecoxib in rat appeared to be comparable to the clinical dose in humans, whereas the dose of 200 mg/kg celecoxib in rats corresponded to a supra-therapeutic dose in humans. In a previous report, when the dose of celecoxib was increased up to 400 mg/kg in rats, the Cmax was reported to be 17.7 μM (Paulson et al., 1999), which was comparable with our results. In these reports, the celecoxib concentrations did not reach the Ki value even at supra-clinical dosage.

In general, the interaction between methotrexate and NSAIDs is known to involve multiple processes. In addition to the inhibition of methotrexate tubular

secretion by NSAID, It has been reported that NSAID decreased glomerular filtration of methotrexate via a reduction of renal blood flow with inhibition of prostaglandin and the NSAID also competed for protein-binding sites of methotrexate (Ahern et al., 1988; Brouwers and de Smet, 1994). However, we have previously found that contributions of these two factors were relatively small for pharmacokinetic interaction and competition of renal tubular secretion was the major factor to determine the pharmacokinetics. hOAT3 is the main transporter at basolateral membrane for uptake of methotrexate from the blood fluid and its mRNA expression level is the highest among the OAT family (Motohashi et al., 2002; Takeda et al., 2001). However, all mechanisms of pharmacokinetic interaction cannot be explained merely by the competition for hOAT3. It is necessary to consider the contribution of other transporters of these drugs at the apical membrane of the kidney tubule. (Shibayama et al., 2006; Takeuchi et al., 2001; Van Aubel et al., 2000). Furthermore, it is also necessary that the contribution of the interaction between methotrexate and celecoxib metabolites is considered. Celecoxib is rapidly metabolized in rats via oxidative pathway and almost excreted in the feces within the 24h (Paulson, Zhang et al, 2000). The metabolism of celecoxib in rats is similar to the humans occurring primarily through a single metabolic pathway.

There is a difference on substrate specificity between hOAT3 and rOAT3 (Cha et al. 2001: Hasazawa et al. 2002). We did not have any data about direct comparison between hOAT3 and rOAT3 in celecoxib/MTX transport and we think this is a limitation of this study. However, in our previous study, serum methotrexate concentrations were increased in the rat when concentrations of NSAIDs were more than the Ki values obtained by hOAT3-uptake studies. Thus,

our in vitro data with hOAT3-expressing cells can be used in evaluating renal pharmacokinetic interactions in rats. This finding is compatible with a previous report that the character of OAT3 in humans is comparable to that in rats (Matsumoto et al. 2007). We think this in vitro study provides useful information when we consider the pharmacokinetic drug interaction in humans.

In conclusion, celecoxib inhibited the methotrexate tubular secretion via OAT3 and this result suggested that celecoxib is a substrate of hOAT3. However, celecoxib did not show any pharmacokinetic interaction with methotrexate in vivo in clinical dosage mainly because serum concentrations of the drug was smaller than the Ki value obtained from the in vitro study. To predict the magnitude of the pharmacokinetic interaction between methotrexate and the new drug by in vitro study, it is necessary to obtain not only the Ki value by in vitro study but also the drug's therapeutic concentrations. It could be presumed that any drug administered at a therapeutic concentration less than the Ki value will show little interaction with methotrexate. Therefore, our accumulation study using S2-hOAT3 cells might be useful for screening potential interactions between methotrexate and new drugs *in vivo*, provided that inhibitory concentration can be achieved in vivo.

5. Acknowledgment

We thank Dr. George J. Schwartz (Department of Pediatrics, University of Rochester, U.S.A.) for critical reading of the manuscript. Part of this study was supported by Grant from Ministry of Education, Culture, Sports, Science and Technology of Japan.

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FIGURE LEGENDS

Fig. 1. Effects of celecoxib and other NSAIDs on [^{3}H]methotrexate accumulation in S2-hOAT3 cells. The results are presented as the means ± S.D. (n =6). *P < 0.01 vs. vehicle.

Fig. 2. Serum methotrexate concentrations after intraperitoneal injection of methotrexate. The results of each plot are presented as the means ± S.D. (n = 6). Celecoxib 50 mg/kg (●), 200 mg/kg (○); sulindac 50 mg/kg (▲); acetaminophen 400 mg/kg (△); vehicle (■). *P < 0.01 vs. vehicle.

Fig. 3. Celecoxib concentrations in serum after intraperitoneal injection of methotrexate. The results of each plot are presented as the means ± S.D. (n = 6): celecoxib 50 mg/kg (●), 200 mg/kg (○).

Fig. 4. Percentage of methotrexate excreted in urine. The results are presented as the means ± S.D. *P < 0.05, **P < 0.01 vs. vehicle.

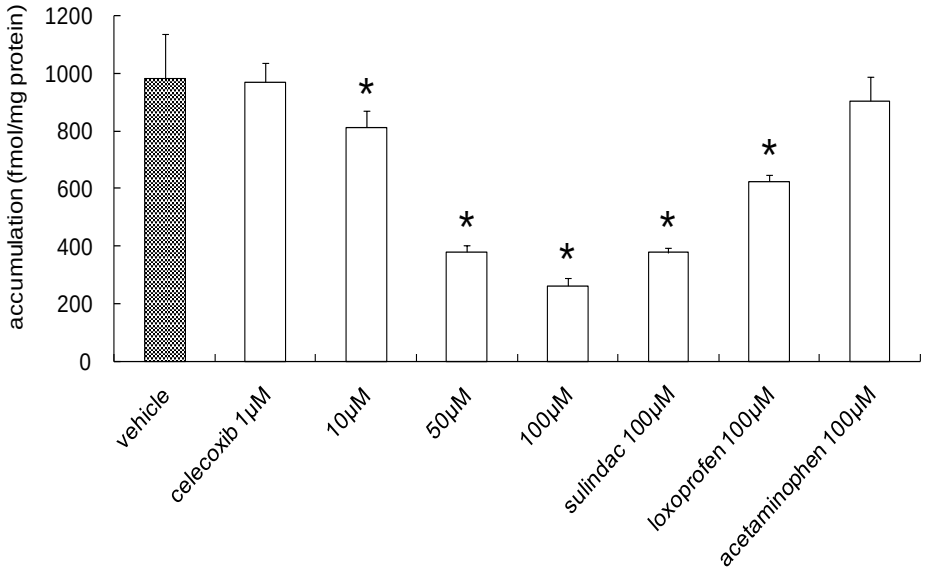
accumulation (fmol/mg protein)
1200
1000
800
600
400
200
0
*
*
*
*
*
vehicle
celecoxib 1μM
10μM
50μM
100μM
sulindac 100μM
loxoprofen 100μM
acetaminophen 100μM

Fig.1

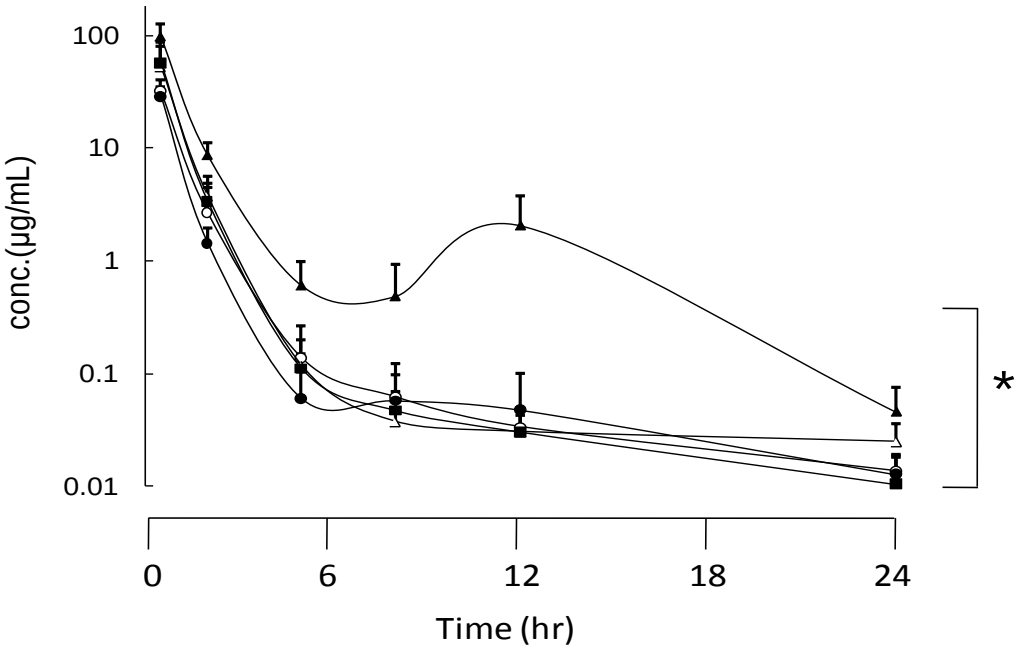

Fig.2

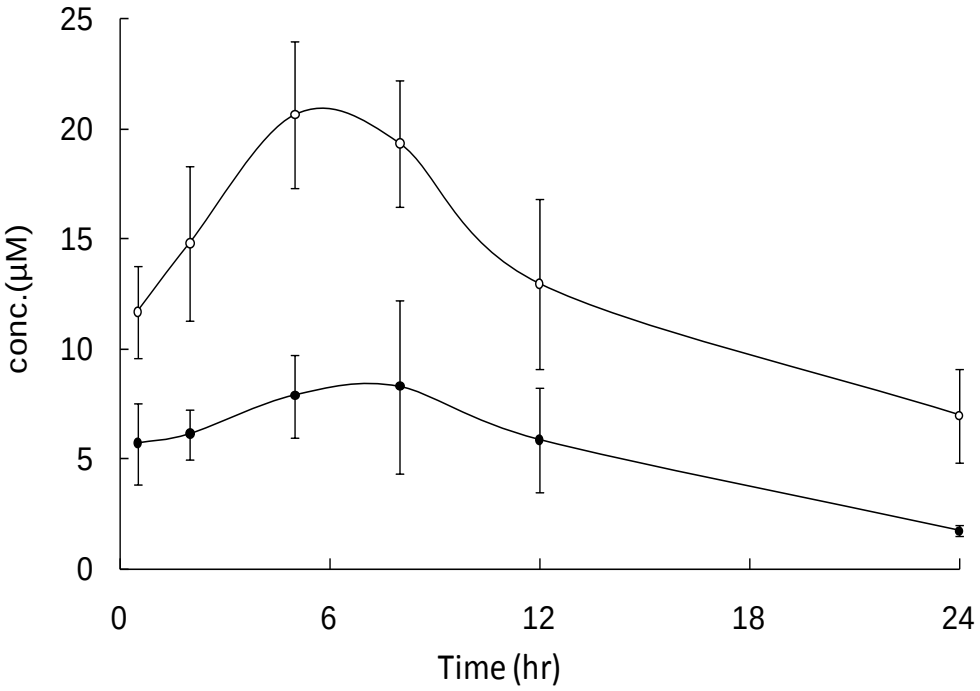

Fig.3

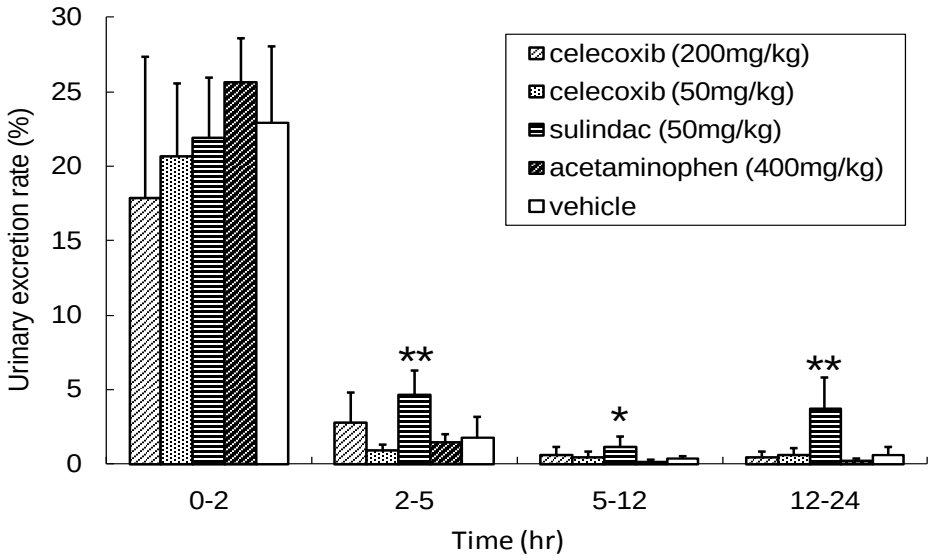
30
25
20
15
10
5
0
Urinary excretion rate (%)
celecoxib (200mg/kg)
celecoxib (50mg/kg)
sulindac (50mg/kg)
acetaminophen (400mg/kg)
vehicle
**
*
**
0-2
2-5
5-12
12-24
Time (hr)

Fig.4