Theophylline, 1,3-dimethylxanthine, has been widely used as a bronchodilatory drug for the treatment of neonatal apnea in premature newborns and patients with chronic obstructive airway disease such as asthma and bronchitis. Theophylline is metabolized by the liver and kidneys. Theophylline metabolism is predominantly via demethylation to 1-methylxanthine and 3-methylxanthine and by 8-hydroxylation to 8-hydroxytheophylline. Theophylline is eliminated primarily via the kidneys, but the liver also plays a role in its elimination.

Macrolide antibiotics such as erythromycin have been used for treatment of a variety of infections and are often combined with other drugs. For example, patients with chronic asthma receiving continuous therapy with theophylline may require short courses of erythromycin for unrelated pyogenic infections. It has been reported that theophylline and erythromycin may interact, and this interaction could be mediated by drug transporters.

In the present study, we investigated the possible involvement of hOat2 in the interaction of theophylline with erythromycin.

**Materials and Methods**

**Chemicals.** [14C]Theophylline (52 mCi/mmol) and [14C]erythromycin (55 mCi/mmol) were purchased from American Radiolabeled Chemicals. Xenopus laevis Oocyte Preparation and cRNA Synthesis. Isolation and preparation of Xenopus oocytes was performed as described elsewhere (Kobayashi et al., 2002b). The hOat2 cDNA was linearized with BamHI, and the capped cRNA was transcribed in vitro by T7 RNA polymerase. Defolliculated oocytes were microinjected with 50 ng of in vitro transcribed cRNA under a stereomicroscope using a microdispenser.

**Transport Assays.** Uptake experiments of radiolabeled substrates were performed in an ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, pH 7.4) at room temperature. Oocytes were incubated in an ND96 solution containing radiolabeled substrates for 1 h at room temperature. For the kinetic study, concentration-dependent uptake experiments of [14C]theophylline and [14C]erythromycin were performed with each compound at a final concentration ranging from 1, 2, 5, 10, 15, and 20 μM, and 1, 5, 10, 20, 40, and 60 μM, respectively. The compounds were incubated with oocytes expressing hOat2 for 1 h at room temperature. The uptake was terminated by the addition of 2 ml of an ice-cold ND96 solution.
solution, and the oocytes were washed with the same solution at least five times. Counts in control (noninjected) oocytes were subtracted from the counts in cRNA-injected oocytes. The oocytes were solubilized with 250 μl of 10% SDS, and accumulated radioactivity was determined with a liquid scintillation counter. *K*ₘ indicates the Michaelis-Menten constant (micromolar). We repeated each experiment more than three times to confirm the results.

**Inhibition Study.** For the inhibition study, oocytes expressing hOat2 were incubated for 1 h in an ND96 solution containing [14C]theophylline (10 μM) or [14C]erythromycin (10 μM) in the presence or absence of inhibitors at a final concentration of 1 mM. Theophylline, erythromycin, clarithromycin, and valproate were directly dissolved in an ND96 solution from a stock solution. These stock solutions of the inhibitors were prepared in dimethyl sulfoxide and diluted to a final concentration as described above. The final concentration of dimethyl sulfoxide in the assay medium did not exceed 1.0%.

**Statistical Analysis.** Statistical differences were determined by the unpaired Student’s *t* test. Comparisons of data measuring initial rates of uptake of radiolabeled substrates in the presence and absence of inhibitors were determined by analysis of variance. The values represent the mean ± S.E.M. (*p* < 0.05).

**Results and Discussion**

This paper describes a possible involvement of hOat2 [SLC22A7] on the interaction of theophylline with erythromycin using a *Xenopus* oocyte expression system. Theophylline has been widely used for the treatment of asthma and chronic obstructive pulmonary disease. Pharmacokinetic studies have revealed that orally administered theophylline is almost completely absorbed, and the liver is responsible for biotransformation of about 90% of theophylline (Cornish and Christman, 1957; Cummins et al., 1976; Kozak et al., 1977; Pfeifer et al., 1979). It has been reported that theophylline clearance is potentially modified by many factors, including hepatic microsomal P450-mediated demethylation, xanthine oxidase, or hepatic uptake (Jonkman and Upton, 1984).

On the other hand, the macrolide antibiotic erythromycin was derived from *Streptomyces erythreus* in 1952. When acute infectious complications arise, macrolides are frequently used, and erythromycin is often chosen in the clinical setting. In addition, erythromycin has a broad spectrum of activity similar to that of penicillin; therefore, this drug can be chosen for the treatment of infections in patients who are allergic to penicillin.

Several reports suggest that macrolide antibiotics may alter theophylline clearance. The interaction between theophylline and erythromycin would clearly be of clinical importance because of its toxicity. The exact mechanism of interference with theophylline disposition by erythromycin is still unclear; it might be explained by inhibition of the 1-demethylation pathway by erythromycin by inhibiting hepatic CYP1A2 and 3A4 and resulting in an increase of the blood concentration of theophylline (Adebayo et al., 1986; Periti et al., 1992). Because the therapeutic range of theophylline is very narrow, changes in theophylline clearance may result in lack of a sufficient bronchodilating effect or in the appearance of a toxic effect.

Oat2 was initially called the novel liver-specific transport protein (NLT) (Simonson et al., 1994). Oat2, as well as OATP/Oatp, is a multispecific organic anion transporter and is highly expressed at the sinusoidal membrane of the liver (Simonson et al., 1994; Sekine et al., 1998). However, as far as we know, it is not clear at this time whether the theophylline-erythromycin interaction can occur via hepatic drug transporters. Therefore, we focused on the role of hOat2 and assumed that hOat2 could transport theophylline and erythromycin.

Using *Xenopus* oocytes injected with hOat2 cRNA, we first examined the transport of theophylline and erythromycin mediated by hOat2. Since the uptake of [14C]theophylline by uninjected oocytes was equal to that of the oocytes injected with 50 nl of water (data not shown), uninjected oocytes were used as the control instead of water-injected oocytes throughout this study. As shown in Fig. 1, both [14C]theophylline and [14C]erythromycin were significantly transported via hOat2 to about 5.9-fold and 33.3-fold that of the controls, respectively. These compounds are identified for the first time as hOat2 substrates. We have revealed that mouse Oat2 (mOat2) is a multispecific organic anion transporter (Kobayashi et al., 2002b).

To determine the affinity of theophylline and erythromycin with hOat2, based on these findings, concentration dependence of the uptake of [14C]theophylline and [14C]erythromycin via hOat2 was subsequently examined. As shown in Fig. 2, hOat2 mediated the transport of [14C]theophylline, and [14C]erythromycin showed saturable kinetics and followed the Michaelis-Menten equation. Eadie-Hofset plots of the concentration dependence of theophylline and erythromycin uptake after subtraction of the uptake by noninjected oocytes revealed that the estimated *K*ₘ values are 12.6 μM and 18.5 μM, respectively. Thus, the affinities of both compounds with hOat2 are very similar. These results indicate that theophylline and erythromycin are transported via hOat2.

The therapeutic range of plasma concentration and unbound fraction of theophylline were reported to be 55 to 110 μM and 40 to 60%, respectively (Tenenbein, 1989). Therefore, the steady-state concentration of unbound theophylline in the plasma is estimated to be approximately 41.3 μM. In addition, the maximum plasma concentration and unbound fraction of erythromycin were reported to be 4.8 μM and 84%, respectively (Periti et al., 1989); the steady-state concentration of unbound erythromycin in the plasma is estimated to be approximately 4.0 μM. Comparing the plasma concentration and our kinetic data, hOat2 may be a responsible molecule for the transport of theophylline and the interaction of theophylline with erythromycin in the human liver.

To confirm hOat2 involvement in the theophylline-erythromycin drug interaction, *cis*-inhibitions of hOat2-mediated drug uptake experiments were performed. The results are shown in Fig. 3. The hOat2-mediated transport of [14C]theophylline was strongly inhibited by erythromycin and slightly inhibited by clarithromycin. No inhibitory effect was observed by valproate. Similarly, transport of [14C]erythromycin via hOat2 was inhibited by unlabeled theophylline, but not by clarithromycin and valproate. Although some reports were...
unable to confirm any significant effects of theophylline-erythromycin drug interaction (Kelly et al., 1981; Melethil et al., 1982), our present findings, together with the membrane localization, suggest that theophylline-erythromycin drug interaction may occur at the sinusoidal membrane of the liver by inhibiting hOat2-mediated transport.

Regarding the interaction between theophylline and clarithromycin, von Rosensteli and Adam (1995) have reported that there is a slight increase in serum concentration of theophylline AUC and a 16.4% decrease in theophylline clearance with concurrent administration of both drugs. Supportive evidence has been reported by Gillum et al. (1996). Taking their reports (von Rosensteli and Adam, 1995; Gillum et al., 1996) and our inhibition experiment into consideration, theophylline-clarithromycin interaction would be rare, or if it occurred, such interaction may not be mediated by hOat2. Further study is required.

The expression of Oat isoforms (Oat1–Oat3) exhibits a sex difference in rats and mice (Buist et al., 2002; Kobayashi et al., 2002a,b; Buist and Klaassen, 2004). Rat Oat2 in the liver is expressed at a higher level in male rats than in females (Buist et al., 2002; Kobayashi et al., 2002a), whereas mOat2 mRNA of the liver is detected predominantly in females rather than in males (Kobayashi et al., 2002b). Recently, Buist and Klaassen (2004) have extensively investigated the theory that there is no sex-related differential gene expression of mOat2 in the liver using branched DNA analysis. Although there are no reports concerning the gender-related differential expression of hOat2 mRNA in the liver of men and women, Nafziger and Bertino (1989) have reported that theophylline is cleared significantly higher in women than in men, suggesting that such sex-dependent theophylline clearance might be caused by the differential gene expression of hOat2 between men and women. In this respect, additional study is needed.

In conclusion, our paper is the first report concerning the possible involvement of hOat2 on theophylline-erythromycin interaction. Our present findings, therefore, indicate that hOat2 may, at least partly, be involved in the interaction of theophylline with erythromycin at the sinusoidal membrane of the human liver. It would be interesting to elucidate whether other transporters such as OATP1B1, 1B3, and 2B1 (Kool et al., 1999; Hagenbuch and Meier, 2004) are also involved in the theophylline-erythromycin interaction.

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