

# Hepatic Uptake of the Magnetic Resonance Imaging Contrast Agent Gadoxetate by the Organic Anion Transporting Polypeptide Oatp1<sup>1</sup>

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## ABSTRACT

Gadoxetate is a new hepatobiliary magnetic resonance imaging contrast agent. It is specifically taken up by hepatocytes, and its uptake can be inhibited by the coadministration of bromosulphophthalein, suggesting an involvement of one or several of the cloned organic anion transporting polypeptides Oatp1, Oatp2, and/or OATP. In this study, we demonstrated saturable uptake of gadoxetate by Oatp1 cRNA-injected *Xenopus laevis* oocytes ( $K_m \sim 3.3$  mM). In contrast, gadoxetate was not taken up by Oatp2 or OATP cRNA-injected oocytes. Oatp1-mediated

gadoxetate uptake (100  $\mu$ M) could be inhibited by 10  $\mu$ M bromosulphophthalein (45%), 200  $\mu$ M taurocholate (92%), 100  $\mu$ M rifamycin SV (97%), and 100  $\mu$ M rifampicin (51%). These results show that gadoxetate is a low-affinity substrate of Oatp1. Oatp1-mediated gadoxetate transport demonstrated a similar apparent  $K_m$  value and *cis*-inhibition pattern as previously determined in rats *in vivo*, indicating that Oatp1 is significantly involved in gadoxetate uptake into rat liver.

Gadoxetate (gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid, disodium salt; Fig. 1) is a new hepatobiliary magnetic resonance imaging contrast agent based on the extracellular fluid marker gadopentetate [gadolinium-diethylenetriamine-pentaacetic acid (Magnevist)]. The introduction of the lipophilic ethoxybenzyl moiety to gadopentetate resulted in liver-specific contrast enhancement due to specific uptake into hepatocytes and biliary excretion of gadoxetate (Vogl et al., 1996). Gadoxetate is in phase III of clinical trials and has been evaluated for the detection of liver metastases, hepatocellular carcinomas, hemangiomas, and cholestasis (Schuhmann-Giampieri et al., 1992; Ni et al., 1994; Vogl et al., 1996; Reimer et al., 1997; Schmitz et al., 1997).

Gadoxetate is highly water soluble and exhibits low protein binding (10%) (Weinmann et al., 1991). The substance has a high *in vivo* complex stability (Schuhmann-Giampieri et al., 1992), and there apparently is no biotransformation

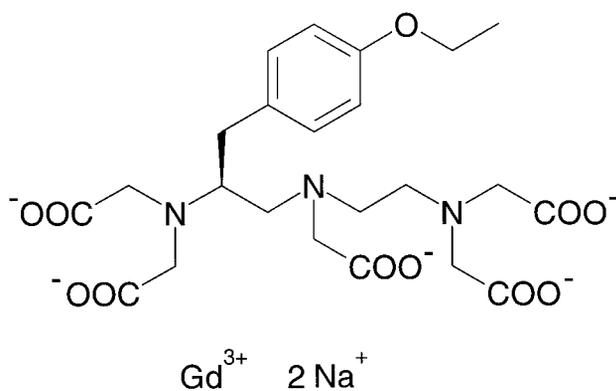
(Weinmann et al., 1991). In rats, 70% of the dose undergoes hepatobiliary excretion, whereas 30% of the dose is excreted in the urine (Weinmann et al., 1991). Gadoxetate exhibits nonlinear pharmacokinetics due to saturability of the hepatobiliary excretion. Renal excretion is linear, and its clearance value is similar to the value for glomerular filtration in rats (Schuhmann-Giampieri et al., 1993b). Experiments in which either the common bile duct or the renal blood vessels were ligated showed that dysfunction of liver or kidney may be fully compensated by the remaining alternative elimination pathway (Muhler et al., 1994).

The biliary excretion of gadoxetate is inhibited by the coadministration of bromosulphophthalein (BSP), which is attributed to a decreased liver uptake of gadoxetate (Clement et al., 1992). This finding suggests that one or several of the polyspecific organic anion transporting polypeptides (Oatps) are involved in the uptake of gadoxetate. The first member of the Oatp gene family, Oatp1, has been isolated from rat liver using expression cloning in *Xenopus laevis* oocytes on the basis of Na<sup>+</sup>-independent BSP uptake (Jacquemin et al., 1994). In addition to BSP, Oatp1 also mediates the uptake of a wide variety of structurally unrelated compounds, including taurocholate and conjugated and neutral steroids (Kullak-Ublick et al., 1994; Bossuyt et al., 1996). Oatp2 has been cloned from rat brain but also is expressed in the liver (Noe

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**ABBREVIATIONS:** BSP, bromosulphophthalein; Oatp, organic anion transporting polypeptide.



**Fig. 1.** Chemical structure of gadoxetate (gadolinium-ethoxybenzyl-diethylenetriamine-pentaacetic acid, disodium salt).

et al., 1997). It has a similar substrate specificity as Oatp1, but as a unique feature, Oatp2 mediates high-affinity uptake of the cardiac glycoside digoxin. OATP has been cloned from human liver. Its substrate specificity is similar to the rat Oatps, although it has lower transport capacities for bile acids and organic anions (Meier et al., 1997). In this study, we investigated whether one or several of the Oatps can mediate the hepatic uptake of gadoxetate with similar characteristics as in intact liver.

## Experimental Procedures

**Materials.** Radiolabeled [ $^{153}\text{Gd}$ ]gadoxetate (107.51 MBq/mg) and unlabeled gadoxetate were kindly provided by Schering AG (Berlin, Germany) (Schuhmann-Giampieri et al., 1992). [ $^3\text{H}$ ]Estrone-3-sulfate (53 Ci/mmol) and [ $^3\text{H}$ ]digoxin (15 Ci/mmol) were obtained from Du Pont-New England Nuclear (Boston, MA). All other chemicals were of analytical grade and were readily available from commercial sources.

**Uptake Studies in *X. laevis* Oocytes.** In vitro synthesis of Oatp1, Oatp2, and OATP cRNA was performed as described previously (Kullak Ublick et al., 1994, 1995; Noe et al., 1997). *X. laevis* oocytes were prepared (Hagenbuch et al., 1990) and cultured overnight at 18°C. Healthy oocytes were microinjected with 1 ng of Oatp1, 5 ng of Oatp2, and/or 2.5 ng of OATP cRNA and cultured for 3 days in a medium containing 88 mM NaCl, 2.4 mM  $\text{NaHCO}_3$ , 1 mM KCl, 0.3 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.41 mM  $\text{CaCl}_2$ , 0.82 mM  $\text{MgSO}_4$ , 0.05 mg/ml gentamycin, and 15 mM HEPES, pH 7.6. The uptake medium consisted of 100 mM NaCl, 2 mM KCl, 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$ , and 10 mM HEPES, pH 7.5. The oocytes were prewashed in the uptake medium and then incubated at 25°C in 100  $\mu\text{l}$  of the uptake medium containing the indicated substrate concentrations. Water-injected oocytes were used as controls for unspecific uptake of the substrate. After the indicated time intervals, uptake was stopped by the addition of 6 ml of ice-cold uptake medium. The oocytes were washed twice with 6 ml of ice-cold uptake medium, and the oocyte-associated radioactivity of [ $^{153}\text{Gd}$ ]gadoxetate was determined in a Packard Cobra Auto-Gamma counter (Canberra Packard, Zürich, Switzerland). In the case of the positive controls [ $^3\text{H}$ ]estrone-3-sulfate and [ $^3\text{H}$ ]digoxin, each oocyte was dissolved in 0.5 ml of 10% SDS and 5 ml of scintillation fluid (Ultima Gold; Canberra Packard), and the oocyte-associated radioactivity was determined in a Tri-Carb 2200 CA liquid scintillation analyzer (Canberra Packard). To determine the kinetic constants for Oatp1-mediated gadoxetate uptake, a nonlinear curve-fitting program was used (Systat 6.0.1; SPSS Inc., Chicago, IL) using a simple Michaelis-Menten model ( $v = V_{\text{max}}[\text{S}]/(K_m + [\text{S}])$ ). In the *cis*-inhibition studies, the inhibitors bromosulfophthalein (BSP; sodium salt) and taurocholate (sodium salt) were dissolved in the incubation buffer. Because of their low water solubility, the inhibi-

tors rifamycin SV (sodium salt) and rifampicin were dissolved in dimethyl sulfoxide and subsequently diluted 1:100 in the incubation medium to the desired concentration. As a control, Oatp1 cRNA-injected oocytes were also incubated in 1% dimethyl sulfoxide and showed normal gadoxetate uptake.

**Statistical Analysis.** Uptake results are given as mean  $\pm$  S.D. Statistical significance was determined using the Mann-Whitney *U* test (Systat 6.0.1, SPSS Inc., Chicago, IL).

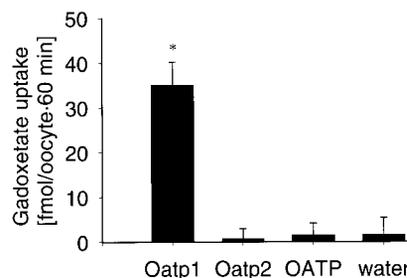
## Results

The uptake of gadoxetate (5  $\mu\text{M}$ ) was measured in Oatp1, Oatp2, and OATP cRNA-injected *X. laevis* oocytes. As illustrated in Fig. 2, only Oatp1 cRNA-injected oocytes mediated significant gadoxetate uptake. The gadoxetate uptake was approximately 22-fold higher in Oatp1 cRNA-injected oocytes compared with water-injected control oocytes. Gadoxetate uptake by Oatp2 and OATP cRNA-injected oocytes was not different from water-injected control oocytes. Proper expression of the carriers in the oocytes was controlled with the positive controls estrone-3-sulfate (0.2  $\mu\text{M}$ ) for Oatp1 and OATP and digoxin (0.8  $\mu\text{M}$ ) for Oatp2, respectively (Meier et al., 1997) (data not shown).

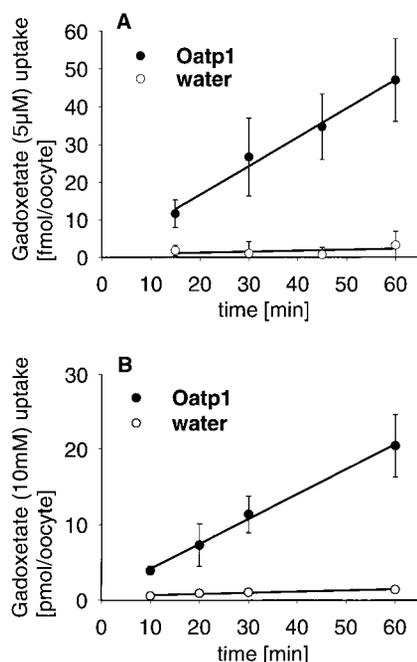
In time-course experiments, the uptake of gadoxetate by Oatp1 was measured during 60 min at the lowest (5  $\mu\text{M}$ ) and the highest (10 mM) concentration used in all uptake experiments (Fig. 3). Oatp1-mediated gadoxetate uptake increased linearly during at least 60 min at both concentrations. In all subsequent experiments designed to determine the kinetic parameters for Oatp1-mediated gadoxetate uptake, the oocytes were incubated for 20 min with increasing substrate concentrations.

Uptake of gadoxetate by Oatp1 was saturable and yielded an apparent  $K_m$  value of  $3.3 \pm 0.4$  mM (mean  $\pm$  S.E.) and a  $V_{\text{max}}$  value of  $544 \pm 33$  fmol/oocyte/min (Fig. 4).

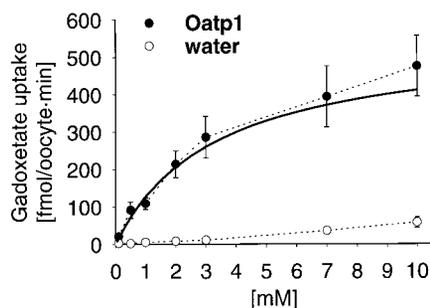
Experiments with rats in vivo showed that BSP inhibited liver uptake of gadoxetate (Clement et al., 1992). Hepatic uptake of gadoxetate was also effectively blocked by the anions rifamycin and bilirubin (Weinmann et al., 1996). Based on these in vivo inhibition data, a series of *cis*-inhibition studies were performed to further confirm the importance of Oatp1 in liver uptake of gadoxetate (Fig. 5). An excess of 10 mM cold gadoxetate inhibited Oatp1-mediated uptake of gadoxetate (100  $\mu\text{M}$ ) by  $79 \pm 8\%$  (mean  $\pm$  S.D.). The known Oatp1 substrates BSP (10  $\mu\text{M}$ ) and taurocholate (200  $\mu\text{M}$ ) inhibited gadoxetate uptake by  $45 \pm 11\%$  and  $92 \pm$



**Fig. 2.** Comparison of Oatp1-, Oatp2-, and OATP-mediated gadoxetate uptake by *X. laevis* oocytes. *X. laevis* oocytes were injected with 1 ng of Oatp1, 5 ng of Oatp2, and 2.5 ng of OATP cRNA. After 3 days in culture, uptake of 5  $\mu\text{M}$  gadoxetate was measured at 60 min (see *Experimental Procedures*). Data represent the mean  $\pm$  S.D. of 14 to 16 oocyte uptake measurements in one representative preparation of two separate oocyte preparations. \*Significantly different from water control (Mann-Whitney *U* test,  $p < .001$ ).



**Fig. 3.** Time course of gadoxetate uptake in Oatp1 cRNA injected *X. laevis* oocytes. *X. laevis* oocytes were injected with 1 ng of Oatp1 cRNA. After 3 days in culture, the time courses for 5  $\mu$ M (A) and 10 mM (B) gadoxetate were measured (see *Experimental Procedures*). Data are expressed as mean  $\pm$  S.D. of 9 to 15 oocyte uptake measurements.

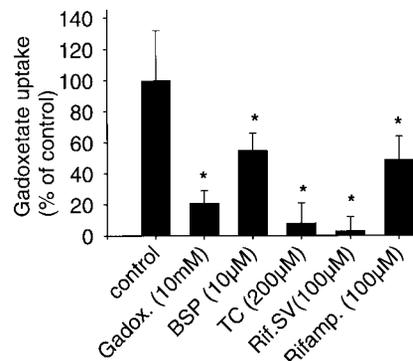


**Fig. 4.** Kinetics of gadoxetate uptake in Oatp1 cRNA-injected *X. laevis* oocytes. *X. laevis* oocytes were injected with 1 ng of Oatp1 cRNA. After 3 days in culture, uptake of gadoxetate was measured at 20 min (see *Experimental Procedures*) at increasing substrate concentrations. The solid line represents uptake differences between Oatp1 cRNA- and water-injected oocytes. Data are expressed as mean  $\pm$  S.D. of 10 to 12 oocyte uptake measurements in two separate oocyte preparations.

13%, respectively. Gadoxetate uptake by Oatp1 was also inhibited by both rifamycin SV (100  $\mu$ M) and its clinically more relevant derivative, rifampicin (100  $\mu$ M), to the extents of 97  $\pm$  9% and 51  $\pm$  15%, respectively.

## Discussion

Gadoxetate is a new magnetic resonance imaging contrast agent that is specifically taken up by hepatocytes and excreted into bile (Schuhmann-Giampieri et al., 1992). During preclinical trials, it was shown that the hepatobiliary excretion of gadoxetate in rats was saturable, suggesting carrier-mediated gadoxetate transport. Recently, several transport proteins with broad substrate specificities have been cloned. The members of the organic cation transporter family (e.g., rOCT1, rOCT2) transport small organic cations (Koepsell, 1998; Zhang et al., 1998). The organic anion transporters



**Fig. 5.** The *cis*-inhibition of gadoxetate uptake in Oatp1 cRNA-injected *X. laevis* oocytes. *X. laevis* oocytes were injected with 1 ng of Oatp1 cRNA. After 3 days in culture, uptake of gadoxetate (100  $\mu$ M) was measured at 20 min (see *Experimental Procedures*) in the absence (control) and the presence of 10 mM gadoxetate (Gadox.), 10  $\mu$ M BSP, 200  $\mu$ M taurocholate (TC), 100  $\mu$ M rifamycin SV (Rif.SV), and 100  $\mu$ M rifampicin (Rifamp.). Data are expressed as mean  $\pm$  S.D. of 13 or 14 oocyte uptake measurements. \*Significantly different from water control (Mann-Whitney *U* test,  $p < .001$ ).

OAT1 and OAT2 mediate mainly the transport of small anionic drugs (molecular weight in the range of 150–350), such as *p*-aminohippurate, salicylate, and acetylsalicylate (Sekine et al., 1997, 1998; Sweet et al., 1997). In contrast, the Oatps mediate the transport of larger, amphipathic molecules, such as taurocholate and BSP (Meier et al., 1997). Gadoxetate is a rather large (molecular weight 726) dianion, and its hepatic uptake could be inhibited by the coadministration of BSP (Schuhmann-Giampieri et al., 1992). Furthermore, uptake of gadoxetate by rat hepatocytes could be inhibited by the anions bilirubin and rifamycin (Weinmann et al., 1996). These findings suggested the involvement of one or several of the cloned Oatps because BSP uptake was shown to be mediated by Oatp1 and OATP (Meier et al., 1997). In this study, it was tested whether the uptake of gadoxetate by hepatocytes is mediated by Oatp1, Oatp2, and/or OATP.

This study shows that only Oatp1 cRNA-injected *X. laevis* oocytes mediated significant gadoxetate uptake activity (Fig. 2). This finding is further proof for the functional differences between the members of the Oatp gene family (Meier et al., 1997). Because gadoxetate is negatively charged at physiological pH, exclusive uptake by Oatp1 further supports the preference of Oatp1 for organic anions. In contrast, the unique feature of Oatp2 is high-affinity uptake of the neutral cardiac glycoside digoxin (Noe et al., 1997), whereas OATP exhibits high initial uptake rates for the cation *N*-(4,4-azo-*n*-pentyl)-21-deoxyajmalinium and the thrombin inhibitor CRC 220 (Meier et al., 1997).

Oatp1-mediated gadoxetate uptake exhibited saturability with increasing substrate concentrations (Fig. 4). The apparent  $K_m$  value was 3.3  $\pm$  0.4 mM (mean  $\pm$  S.E.). Nonlinear pharmacokinetic modeling of the gadoxetate plasma concentration time course and the urinary and biliary gadoxetate excretion data after i.v. injection of a gadoxetate bolus (0.5 mmol/kg) in rats yielded a  $K_m$  value of 2.7  $\pm$  1.45 mM (mean  $\pm$  S.D.) (Schuhmann-Giampieri, 1993a). This similarity between the  $K_m$  value of Oatp1-mediated gadoxetate uptake and the  $K_m$  value of fitted *in vivo* data indicates that Oatp1 is significantly involved in gadoxetate uptake into rat liver. There is no saturation of the hepatobiliary elimination pathway with diagnostic doses of gadoxetate (0.01–0.03

mmol/kg; Weinmann et al., 1991) because saturation of the hepatobiliary elimination pathway is reached only with doses of more than 0.5 mmol/kg (Schuhmann-Giampieri et al., 1992). Hence, the involvement of Oatp1 as a low-affinity, high-capacity carrier would be favorable for rapid liver enhancement because sufficient amounts of gadoxetate can efficiently be taken up by the hepatocytes. Apart from biliary elimination, gadoxetate may regurgitate from the hepatocytes into the bloodstream and then is cleared by the kidney (Muhler et al., 1994). The efflux of gadoxetate back to the blood could also be mediated by Oatp1 because it was shown that Oatp1 can mediate bidirectional BSP transport in stably transfected HeLa cells (Shi et al., 1995).

The *cis*-inhibition studies further support the hypothesis that Oatp1 is an important uptake carrier for gadoxetate in rat liver. Similar to the *in vivo* situation (Weinmann et al., 1991; Schuhmann-Giampieri et al., 1992), Oatp1-mediated gadoxetate uptake was inhibited by BSP and rifampicin SV (Fig. 5). The clinically more relevant drug rifampicin also inhibited gadoxetate uptake by Oatp1. Kullak-Ublick et al. (1994) reported previously that rifampicin does not alter Oatp1-mediated BSP uptake. In contrast, Oatp1-mediated gadoxetate uptake was substantially inhibited by rifampicin (Fig. 5). The most probable reasons for this apparent inconsistencies are the different affinities of gadoxetate ( $K_m \sim 3.3$  mM) and BSP ( $K_m \sim 1.5$   $\mu$ M) for Oatp1. Thus, it can be expected that any inhibitor with an Oatp1 affinity between that of BSP and gadoxetate will affect gadoxetate transport but will have little influence on BSP transport.

The finding that the clinically important drug rifampicin inhibits Oatp1-mediated gadoxetate uptake raises the question of whether the coadministration of rifampicin could substantially reduce gadoxetate-induced liver enhancement. In rats, the structurally related rifampicin significantly inhibited hepatic gadoxetate uptake at a dose of 30 mg/kg b.wt. (Weinmann et al., 1996). Extrapolation of these results to humans is not yet possible because the transport protein responsible for gadoxetate uptake into human liver is still unknown. However, the usual rifampicin dose in men is considerably less (9 mg/kg b.wt.), and rifampicin serum and hepatic tissue concentrations in the first hours after administration of therapeutic doses were reported to be in the range of 1 to 14  $\mu$ M and 1 to 52  $\mu$ M, respectively (Kiss et al., 1978). Furthermore, rifampicin is bound to serum proteins to an extent of about 80% (Acocella, 1978), resulting in a free plasma concentration between 0.2 and 3  $\mu$ M. A rifampicin concentration of 100  $\mu$ M in the absence of any serum proteins resulted in a 51% inhibition of Oatp1-mediated gadoxetate uptake (Fig. 5). Thus, if one assumes that the free plasma concentration of rifampicin governs uptake inhibition *in vivo*, no substantial reduction of liver enhancement would be expected after the administration of therapeutic rifampicin doses.

In contrast to several *in vivo* observations in which taurocholate and tauroglycocholate did not interfere with hepatic uptake and biliary excretion of gadoxetate (Clement et al., 1992; Schuhmann-Giampieri et al., 1992, 1993b), taurocholate inhibited Oatp1-mediated gadoxetate transport into *X. laevis* oocytes (Fig. 5). This discrepancy arises because in the oocyte experiments, high taurocholate concentrations (200  $\mu$ M) were used. This value corresponds to four times the apparent  $K_m$  value for Oatp1-mediated taurocholate trans-

port (Kullak-Ublick et al., 1994). For the *in vivo* experiments, no data on taurocholate serum concentrations are available. Because bile acids are very efficiently removed from the circulation, one would expect serum taurocholate concentrations to be only moderately increased. However, if these concentrations remained below the  $K_m$  value for Oatp1-mediated taurocholate transport, one would expect gadoxetate uptake to not be substantially influenced.

In conclusion, the present study shows that Oatp1 is an important carrier for gadoxetate uptake into rat hepatocytes and that gadoxetate is a predominant or even specific Oatp1 substrate. Of course, it cannot be definitively excluded that a transporter not included in the present study might additionally mediate hepatic gadoxetate uptake. However, because of the close agreement between the characteristics of Oatp1-mediated gadoxetate transport with previous *in vivo* data and the physicochemical properties of gadoxetate, a substantial contribution of other transporters, especially the above-mentioned OCTs and OATs, seems unlikely. Because the human OATP does not transport gadoxetate but gadoxetate is concentrated in human liver (Hamm et al., 1995), an Oatp1 analog must also exist in human liver. This hypothetical human Oatp1 analog remains to be identified, cloned, and functionally characterized. Because of its Oatp1 specificity and the clear signal obtained in the oocyte system, gadoxetate seems to be a feasible substrate for expression cloning of the human Oatp1 analog.

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