

**Interaction of oral antidiabetic drugs with hepatic uptake transporters:  
focus on OATPs and OCT1**

Iouri Bachmakov M.D., Hartmut Glaeser Ph.D., Martin F. Fromm M.D., Jörg König Ph.D.

Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-  
University Erlangen-Nuremberg, Fahrstrasse 17, 91054 Erlangen, Germany

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**Corresponding Author:**

Dr. Jörg König

Institute of Experimental and Clinical Pharmacology and Toxicology,  
Friedrich-Alexander-University Erlangen-Nuremberg,  
Fahrstrasse 17, 91054 Erlangen, Germany  
Joerg.Koenig@pharmakologie.med.uni-erlangen.de

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## ABSTRACT

*Objective:* The uptake of drugs into hepatocytes is a key determinant for hepatic metabolism, intrahepatic action as well as their subsequent systemic plasma concentrations and extrahepatic actions. *In vitro* and *in vivo* studies indicate that many drugs used for treatment of cardiovascular diseases (e.g. oral antidiabetic drugs, statins) are taken up into hepatocytes by distinct organic anion transporters (OATPs = organic anion transporting polypeptides; gene symbol *SLCO/SLC21*) or organic cation transporters (OCTs = organic cation transporters; gene symbol *SLC22*). Since most patients with diabetes type 2 receive more than one drug and inhibition of drug transporters has been recognized as a new mechanism underlying drug-drug interactions, we tested the hypothesis whether oral antidiabetic drugs can inhibit the transport mediated by hepatic uptake transporters.

*Research Design and Methods:* Using stably transfected cell systems recombinantly expressing the uptake transporters OATP1B1, OATP1B3, OATP2B1, or OCT1 we analyzed, whether the antidiabetic drugs repaglinide, rosiglitazone, or metformin influence the transport of substrates and drugs [for OATPs: BSP (sulfobromophthalein) and pravastatin; for OCT1: MPP<sup>+</sup> (1-Methyl-4-phenylpyridinium) and metformin].

*Results:* Metformin did not inhibit the uptake of OATP- and OCT1-substrates. However, OATP-mediated BSP and pravastatin uptake as well as OCT1-mediated MPP<sup>+</sup> and metformin uptake was significantly inhibited by repaglinide (IC<sub>50</sub>: 1.6 to 5.6 μM) and rosiglitazone (IC<sub>50</sub>: 5.2 to 30.4 μM).

*Conclusions:* These *in vitro* results demonstrate that alterations of uptake transporter function by oral antidiabetics have to be considered as potential mechanisms underlying drug-drug interactions.

Patients with type 2 diabetes are commonly treated with more than one drug. It is therefore essential to understand mechanisms underlying drug-drug interactions, which might cause changes in the pharmacokinetics and effects of these drugs. It is now well established that drug transporters are major determinants of drug disposition and effects (1; 2). Moreover, inhibition or induction of drug transporters are newly recognized mechanisms of drug-drug interactions (3-5). Oral antidiabetic drugs need to be taken up from the portal venous blood via the basolateral membrane into hepatocytes before they are metabolized in the cell, cause drug effects via intrahepatic mechanisms or are further transported back into the systemic circulation for extrahepatic effects (6-9). Recent *in vitro* and *in vivo* studies highlighted that the transporter-mediated uptake of oral antidiabetic drugs is an important determinant for their disposition and effects (9-11). These uptake mechanisms are also important for disposition and action of a broad variety of cardiovascular drugs frequently used concomitantly in patients with type 2 diabetes (e.g. statins, ACE inhibitors, AT<sub>1</sub> receptor blockers) (12).

In the focus of this study are uptake transporters belonging to the OATP (organic anion transporting polypeptides; gene symbol SLCO/SLC21) and OCT (organic cation transporters; gene symbol SLC22) families. Members of the OATP family transport a variety of anionic endogenous substances and drugs including HMG-CoA reductase inhibitors such as fluvastatin, pitavastatin, pravastatin, and rosuvastatin (12; 13). Furthermore, it has been shown that glitazones and repaglinide may be substrates for the hepatocyte-specific OATP-family member OATP1B1 (9; 14). OATP1B1, OATP1B3, and OATP2B1 are localized in the basolateral membrane of human hepatocytes mediating the uptake of endogenous substances and drugs from the portal venous blood (15-17).

Members of the OCT family play essential roles in the handling of cationic drugs and endogenously synthesized organic cations. Human OCT1 is expressed primarily in the liver, localized in the basolateral membrane of hepatocytes mediating the hepatic uptake of several cationic drugs (e.g. metformin). In addition, various drugs (e.g. cimetidine, desipramine, midazolam, citalopram, clonidine) have been identified to inhibit OCT1-mediated uptake (18).

In spite of the increasingly recognized role of hepatic uptake transporters for drug disposition, it has not been studied systematically whether the oral antidiabetic drugs repaglinide, rosiglitazone, and metformin which have previously been shown to interact with OATPs or OCTs (9; 14; 18) are inhibitors of hepatic uptake transport proteins. To gain more insights into the potential role of uptake transporters for antidiabetic drug mediated drug-drug interactions, we used cell systems stably expressing the human uptake transporters OATP1B1, OATP1B3, OATP2B1, or OCT1 and tested the influence of these oral antidiabetics on the uptake of OATP substrates as well as OCT1 substrates.

## RESEARCH DESIGN AND METHODS

### *Chemicals and antibodies.*

[<sup>3</sup>H]Sulfobromophthalein ([<sup>3</sup>H]BSP; 7585 GBq/mmol) was obtained from Hartmann Analytic (Braunschweig, Germany). [<sup>3</sup>H]1-Methyl-4-phenylpyridinium ([<sup>3</sup>H]MPP<sup>+</sup>; 85 Ci/mmol), [<sup>3</sup>H]rosiglitazone (50 Ci/mmol) and unlabeled rosiglitazone were obtained from BIOTREND Chemikalien GmbH (Cologne, Germany). Unlabeled metformin and poly-D-lysine hydrobromide were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Unlabeled repaglinide was obtained from Chemos GmbH (Regenstauf, Germany). [<sup>14</sup>C]Metformin hydrochloride (0.1 Ci/mmol) was kindly provided by Merck KGaA (Darmstadt, Germany). Methanol (hypergrade), *n*-hexane (p.a.), acetonitrile (hypergrade), and acetic acid (supra pure)

were purchased from Merck KGaA (Darmstadt, Germany). Diethyl ether (99.8 % purity), ammonium acetate (p.a.), and ibuprofen were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

**Cell culture.** Stably transfected human embryonic kidney cells (HEK293 cells) and Madin-Darby canine kidney cells (MDCKII cells) were cultured as described previously (5; 19). For uptake and inhibition experiments stably transfected HEK293 cells recombinantly expressing human OATP1B1 (HEK-OATP1B1), human OATP1B3 (HEK-OATP1B3) (5), or human OATP2B1 (HEK-OATP2B1) and the respective HEK-Control cells (HEK-Co/418; transfected with the empty expression vector pcDNA3.1 or HEK-Co/Hy; transfected with the empty expression vector pcDNA3.1/Hygro) were seeded in poly-D-lysine (0.1mg/ml)-coated 12-well plates (Cell Culture Multiwell Plate, Cellstar, Greiner Bio-One) at an initial density of 700,000 cells/well. The cells were grown to confluence for 1 day and then induced with 10 mM sodium butyrate (Merck KGaA) for 24 h before the uptake experiments to obtain higher levels of the recombinant protein (20).

MDCKII cells were transfected with the respective plasmid pcDNA3.1/Hygro(-)-OCT1 containing the full length cDNA encoding the human OCT1-protein (NM\_003057) using Effectene transfection reagent (Qiagen GmbH, Hilden, Germany). After hygromycin (800 µg/ml) selection, single colonies were characterized for *SLC22A1* (encoding human OCT1) mRNA expression by real-time PCR analysis. Vector transfected MDCKII-control cells were established by the same method using the respective expression plasmid without insert for transfection.

For MPP<sup>+</sup> uptake experiments MDCKII cells were seeded in poly-D-lysine (0.1 mg/ml)-coated 12-well plates (Cell Culture Multiwell Plate CELLSTAR®, Greiner Bio-One, Frickenhausen, Germany) at an initial density of 700,000 cells per well. The cells

were grown to confluence for 2 days and induced with 10 mM sodium butyrate (Merck KGaA, Darmstadt, Germany) for 24 h prior the uptake experiments (20).

**Uptake Assays.** Before starting the uptake experiments with the HEK cells, cells were washed with prewarmed (37 °C) uptake buffer (142 mM NaCl, 5 mM KCl, 1 mM K<sub>2</sub>HPO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 1.5 mM CaCl<sub>2</sub>, 5 mM glucose, and 12.5 mM HEPES, pH 7.3). [<sup>3</sup>H]BSP was dissolved in uptake buffer and unlabeled BSP was added to the final concentration of 0.05 µM BSP for studies with HEK-OATP1B1 and 1 µM BSP for studies with HEK-OATP1B3 and HEK-OATP2B1, respectively. To characterize repaglinide and rosiglitazone as inhibitors of OATP-mediated uptake, both drugs were added in increasing concentrations (0.01 µM to 100 µM). The cells were incubated with the solution at 37 °C for 10 min as described (5). Afterwards, the cells were washed three times with ice-cold uptake buffer before lysing the cells with 0.2% SDS. The intracellular accumulation of radioactivity was detected by liquid scintillation counting (Perkin Elmer Life Sciences GmbH). For the experiments using pravastatin as substrate for OATP1B1 and OATP1B3, a concentration of 50 µM of pravastatin was used. To test the inhibitory effect of repaglinide and rosiglitazone each drug was added in concentrations of 10 µM and 100 µM. The uptake assay was performed as described above. The intracellular pravastatin concentration was determined by LC/MS/MS analysis as previously described (5).

The uptake experiments with MDCKII-OCT1 cells were carried out in an analogous manner. [<sup>3</sup>H]MPP<sup>+</sup> was dissolved in uptake buffer and unlabeled MPP<sup>+</sup> was added to the final concentration of 30 µM. To characterize repaglinide, rosiglitazone, and metformin as inhibitors of OCT1-mediated uptake, the drugs were added in increasing concentrations of 0.1 to 1000 µM for metformin and 0.1 µM to 100 µM for repaglinide and rosiglitazone, respectively.

For experiments using metformin as substrate of OCT1, [ $^{14}\text{C}$ ]metformin was dissolved in uptake buffer and unlabeled metformin was added to a final concentration of 10  $\mu\text{M}$ . To test the inhibitory effect of repaglinide and rosiglitazone each drug was added in a concentration of 0.1 to 100  $\mu\text{M}$ . All experiments were repeated at least 3 times.

**Data Analysis.** The OATP1B1-, OATP1B3-, OATP2B1-, and OCT1-mediated net uptake was obtained by subtracting the uptake in vector-transfected cells from that in OATP1B1, OATP1B3, OATP2B1, and OCT1 expressing cells. The percentage of uptake inhibition was calculated from control experiments in the absence of antidiabetic drugs (100% uptake). The corresponding  $\text{IC}_{50}$  values for uptake inhibition were calculated by fitting the data to a sigmoidal dose response regression curve (Prism 4.01 2004, GraphPad Software, San Diego, USA). Effects of repaglinide and rosiglitazone on pravastatin uptake in OATP1B1 and OATP1B3 cells were analyzed with unpaired two-tailed  $t$  test (Prism 4.01 2004, GraphPad Software, San Diego, USA). A value of  $P < 0.05$  was required for statistical significance.

## RESULTS

### *Characterization of MDCKII-OCT1 cells.*

To examine the inhibitory potency of antidiabetic drugs on OCT1-mediated uptake, MDCKII cells were stably transfected with the *SLC22A1* cDNA and selected for a high expression of the uptake transporter OCT1. The *SLC22A1* mRNA expression of the selected cell clones was analyzed using quantitative real-time PCR. This analysis demonstrated a high *SLC22A1* mRNA expression in several MDCKII-OCT1 clones compared to vector-transfected cells. The cell clone with the highest mRNA expression was used for further transport experiments using the prototypic tritium-labelled substrate 1-methyl-4-phenylpyridinium ( $[^3\text{H}]\text{MPP}^+$ ).  $\text{MPP}^+$  was shown to be a high affinity substrate for OCT1 with a  $K_m$  value of about

21  $\mu\text{M}$ , which is in accordance with previously published data (18). The uptake experiments (Fig. 1A and B) demonstrated that MDCKII-OCT1 cells were able to mediate uptake of  $\text{MPP}^+$  (50  $\mu\text{M}$ ) into cells with an uptake ratio of 3.7-fold compared to control cells. Furthermore, we confirmed that metformin, previously shown to be a substrate for OCT1 with a  $K_m$  value of 1,470  $\mu\text{M}$  (18), is also transported by the newly established MDCKII-OCT1 cells (Fig. 1C).

**Inhibition of OATPs and OCT1 by antidiabetic drugs.** The results of the inhibition experiments are summarized in Table 1 and Figures 2-4. Metformin did not inhibit OATP1B1-, OATP1B3- or OATP2B1-mediated BSP uptake (Fig. 2) as well as uptake of the OCT1 substrate  $\text{MPP}^+$  (Fig. 4). However, OATP-mediated BSP and pravastatin uptake as well as OCT1-mediated  $\text{MPP}^+$  and metformin uptake was significantly inhibited by repaglinide (Fig. 2-4, Table 1) and rosiglitazone (Fig. 2-4, Table 1).

## DISCUSSION

Patients with type II diabetes have frequently to be treated with more than one drug. Effects of oral antidiabetic drugs depend on the extent of drug absorption from the gut lumen, on metabolism of the drug in the liver and on the extent of its excretion into bile and urine (21). In general, modification of all these processes by a second, concomitantly administered drug can alter the effects of oral antidiabetic drugs (21).

Recently, it was recognized that a broad variety of drugs including many cardiovascular drugs such as statins and angiotensin II-receptor antagonists is transported through biological membranes via specific transport proteins (15; 22-24). For example, the efflux transporter P-glycoprotein, which translocates its substrates from the inside of the cell to the outside (e.g. from the hepatocyte into bile) is a major determinant of drug effects (1). If P-glycoprotein-mediated drug excretion is

inhibited by a second, concomitantly administered compound, drug plasma concentrations increase significantly and may result in drug toxicity (3).

In addition to efflux transporters (e.g. P-glycoprotein), uptake transporters such as the organic anion transporting polypeptides (OATPs) or the organic cation transporters (OCT) are important for drug disposition. For example, they mediate the uptake of multiple cardiovascular drugs from the portal venous blood into the hepatocytes (12; 18). This transport process is an essential prerequisite for subsequent drug metabolism in the hepatocytes.

The goal of our *in vitro* study was to investigate, whether the oral antidiabetic drugs repaglinide, rosiglitazone and metformin are inhibitors of the hepatic uptake transporters of the OATP family (OATP1B1, OATP1B3 and OATP2B1) and of the OCT family member OCT1. Whereas metformin did not affect the function of hepatic uptake transporters, both repaglinide and rosiglitazone were significant inhibitors of organic anion and organic cation transport. The following clinical consequences can result from the observed interaction with repaglinide and rosiglitazone. First, if hepatic uptake of a drug (e.g. pravastatin) is inhibited by repaglinide and rosiglitazone and possibly by further oral antidiabetic drugs, the first step (i.e. uptake into hepatocytes) of biliary drug elimination is blocked. This causes increased plasma concentrations and an increased risk of adverse drug reactions. The importance of an impaired uptake transporter function is convincingly shown for OATP1B1. One polymorphism (c.521T>C) in the gene encoding for OATP1B1 results in a significantly reduced OATP1B1 function (25), a situation which is comparable to the OATP1B1 function in the presence of drugs, which inhibit this uptake transporter. Indeed, several studies showed that subjects carrying this polymorphism with the reduced uptake transporter function have significantly higher plasma concentrations of pravastatin

(26-29). Interestingly, for other drugs no influence of this genetic variation could be observed (30). This could be due to an overlapping substrate spectrum with OATP1B3 possibly compensating for a reduced uptake mediated by a mutated OATP1B1 protein.

A second clinical consequence of decreased hepatic drug uptake is a reduced effect of the affected drug (e.g. pravastatin). This option applies if the drug effect (e.g. for pravastatin: cholesterol-lowering effect) is mediated via mechanisms within the hepatocyte. Indeed, a genetically determined reduced hepatic uptake of pravastatin was recently shown to cause a reduced effect of pravastatin (31-33).

The third possible clinical consequence of inhibition of uptake transporter function are increased extrahepatic effects (e.g. for statins an increased risk of myotoxicity). This reduced benefit (cholesterol-lowering effect) / risk (myotoxicity) ratio for OATP1B1-dependent statins by inhibition of hepatic uptake was recently highlighted in an excellent review by Neuvonen et al. (34).

It should be noted that our study focused on *in vitro* studies on mechanisms of drug interactions with oral antidiabetic drugs. As outlined below, the clinical consequences (e.g. pharmacodynamic consequences) are difficult to predict and require further clinical studies. Another interesting observation of our study was the unexpected stimulation of OATP1B3-mediated pravastatin uptake by rosiglitazone (Fig. 3). Similar observations have been made for other substrates (5), however, the molecular mechanisms underlying this effect are unclear and require further studies.

As drugs reach the portal vein directly after intestinal absorption, the drug concentration in portal venous blood is higher than in the systemic circulation. For estimation of the drug concentrations in portal venous blood we used the method of Ito et al. [Table 1, (35)]. The predicted portal venous blood concentrations for repaglinide and rosiglitazone are in the same low  $\mu\text{M}$  range

as the determined  $IC_{50}$  values for inhibition of hepatic uptake, suggesting that inhibition of hepatic drug transporters might cause clinically relevant drug-drug interactions in humans.

To the best of our knowledge, no clinical studies have so far been conducted to investigate an impact of repaglinide or rosiglitazone on disposition of pravastatin or other OATP substrates in humans. Pharmacodynamic interactions between metformin and rosiglitazone (36; 37) or repaglinide (38) in patients have intensively been investigated. For both rosiglitazone and repaglinide additional effects on glucose metabolism in patients with type II diabetes receiving simultaneously metformin have been shown. Whether these effects can in part be explained by an interaction with hepatic metformin uptake is presently unclear. One study examined the pharmacokinetic interaction between rosiglitazone and metformin in humans (39), however without a significant pharmacokinetic interaction between these drugs.

The question arises, whether our findings on inhibition of uptake transporter function by oral antidiabetic drugs can be used to draw general conclusions regarding drug-drug interactions. Similar to the present data,

macrolid antibiotics were previously shown to cause a relevant inhibition of hepatic uptake transporters (5). Moreover, there is evidence that other oral antidiabetic drugs (glibenclamide) are inhibitors of hepatic uptake transporters (40). Thus, our in vitro data generated in this study indicate that inhibition of uptake transporters by oral antidiabetic drugs is a newly recognized mechanism of potential drug-drug interactions. Our data provide the basis for controlled clinical studies in order to clarify the importance of drug-drug interactions with oral antidiabetic drugs in studies with healthy volunteers and patients.

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## REFERENCES

1. Fromm MF: Importance of P-glycoprotein at blood-tissue barriers. *Trends Pharmacol Sci* 25:423-429, 2004
2. Ho RH, Kim RB: Transporters and drug therapy: implications for drug disposition and disease. *Clin Pharmacol Ther* 78:260-277, 2005
3. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM: Inhibition of P-glycoprotein-mediated drug transport: A unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 99:552-557, 1999
4. Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, Kroemer HK: The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 104:147-153, 1999
5. Seithel A, Eberl S, Singer K, Auge D, Heinkele G, Wolf NB, Dörje F, Fromm MF, König J: The influence of macrolide antibiotics on the uptake of organic anions and drugs mediated by OATP1B1 and OATP1B3. *Drug Metab Dispos*, 35:779-786, 2007
6. Bailey CJ, Turner RC: Metformin. *N Engl J Med* 334:574-579, 1996
7. Balfour JA, Plosker GL: Rosiglitazone. *Drugs* 57:921-930; discussion 931-922, 1999
8. Kajosaari LI, Laitila J, Neuvonen PJ, Backman JT: Metabolism of repaglinide by CYP2C8 and CYP3A4 in vitro: effect of fibrates and rifampicin. *Basic Clin Pharmacol Toxicol* 97:249-256, 2005
9. Niemi M, Backman JT, Kajosaari LI, Leathart JB, Neuvonen M, Daly AK, Eichelbaum M, Kivisto KT, Neuvonen PJ: Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics. *Clin Pharmacol Ther* 77:468-478, 2005
10. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, Sheardown SA, Yue L, Burchard EG, Brett CM, Giacomini KM: Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics. *Clin Pharmacol Ther*, 2007 [epub ahead of print]
11. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, Ianculescu AG, Yue L, Lo JC, Burchard EG, Brett CM, Giacomini KM: Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest* 117:1422-1431, 2007
12. König J, Seithel A, Gradhand U, Fromm MF: Pharmacogenomics of human OATP transporters. *Naunyn Schmiedeberg's Arch Pharmacol* 372:432-443, 2006
13. Hagenbuch B, Meier PJ: The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* 1609:1-18, 2003
14. Nozawa T, Sugiura S, Nakajima M, Goto A, Yokoi T, Nezu J, Tsuji A, Tamai I: Involvement of organic anion transporting polypeptides in the transport of troglitazone sulfate: implications for understanding troglitazone hepatotoxicity. *Drug Metab Dispos* 32:291-294, 2004
15. Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP, Kirchgessner TG: A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J Biol Chem* 274:37161-37168, 1999
16. König J, Cui Y, Nies AT, Keppler D: Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem* 275:23161-23168, 2000
17. König J, Cui Y, Nies AT, Keppler D: A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am J Physiol Gastrointest Liver Physiol* 278:G156-164, 2000
18. Koepsell H, Lips K, Volk C: Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 24:1227-1251, 2007
19. Letschert K, Keppler D, König J: Mutations in the SLCO1B3 gene affecting the substrate

- specificity of the hepatocellular uptake transporter OATP1B3 (OATP8). *Pharmacogenetics* 14:441-452, 2004
20. Cui Y, König J, Buchholz JK, Spring H, Leier I, Keppler D: Drug resistance and ATP-dependent conjugate transport mediated by the apical multidrug resistance protein, MRP2, permanently expressed in human and canine cells. *Mol Pharmacol* 55:929-937, 1999
  21. Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M: Metabolic drug interactions. *Lippincott Williams & Wilkins*, 2000
  22. Hochman JH, Pudvah N, Qiu J, Yamazaki M, Tang C, Lin JH, Prueksaritanont T: Interactions of human P-glycoprotein with simvastatin, simvastatin acid, and atorvastatin. *Pharm Res* 21:1686-1691, 2004
  23. Ishiguro N, Maeda K, Kishimoto W, Saito A, Harada A, Ebner T, Roth W, Igarashi T, Sugiyama Y: Predominant contribution of OATP1B3 to the hepatic uptake of telmisartan, an angiotensin II receptor antagonist, in humans. *Drug Metab Dispos* 34:1109-1115, 2006
  24. Yamashiro W, Maeda K, Hirouchi M, Adachi Y, Hu Z, Sugiyama Y: Involvement of transporters in the hepatic uptake and biliary excretion of valsartan, a selective antagonist of the angiotensin II AT1-receptor, in humans. *Drug Metab Dispos* 34:1247-1254, 2006
  25. Tirona RG, Leake BF, Merino G, Kim RB: Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* 276:35669-35675, 2001
  26. Igel M, Arnold KA, Niemi M, Hofmann U, Schwab M, Lütjohann D, von Bergmann K, Eichelbaum M, Kivistö KT: Impact of the SLCO1B1 polymorphism on the pharmacokinetics and lipid-lowering efficacy of multiple-dose pravastatin. *Clin Pharmacol Ther* 79:419-426, 2006
  27. Mwinyi J, Johne A, Bauer S, Roots I, Gerloff T: Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. *Clin Pharmacol Ther* 75:415-421, 2004
  28. Niemi M, Schaeffeler E, Lang T, Fromm MF, Neuvonen M, Kyrklund C, Backman JT, Kerb R, Schwab M, Neuvonen PJ, Eichelbaum M, Kivistö KT: High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). *Pharmacogenetics* 14:429-440, 2004
  29. Nishizato Y, Ieiri I, Suzuki H, Kimura M, Kawabata K, Hirota T, Takane H, Irie S, Kusuhara H, Urasaki Y, Urae A, Higuchi S, Otsubo K, Sugiyama Y: Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* 73:554-565, 2003
  30. Kalliokoski A, Neuvonen M, Neuvonen PJ, Niemi M: No significant effect of SLCO1B1 polymorphism on the pharmacokinetics of rosiglitazone and pioglitazone. *Br J Clin Pharmacol*, 2007
  31. Kivistö KT, Niemi M: Influence of drug transporter polymorphisms on pravastatin pharmacokinetics in humans. *Pharm Res* 24:239-247, 2007 [epub ahead of print].
  32. Niemi M, Neuvonen PJ, Hofmann U, Backman JT, Schwab M, Lütjohann D, von Bergmann K, Eichelbaum M, Kivistö KT: Acute effects of pravastatin on cholesterol synthesis are associated with SLCO1B1 (encoding OATP1B1) haplotype \*17. *Pharmacogenet Genomics* 15:303-309, 2005
  33. Tachibana-Iimori R, Tabara Y, Kusuhara H, Kohara K, Kawamoto R, Nakura J, Tokunaga K, Kondo I, Sugiyama Y, Miki T: Effect of genetic polymorphism of OATP-C (SLCO1B1) on lipid-lowering response to HMG-CoA reductase inhibitors. *Drug Metab Pharmacokin* 19:375-380, 2004
  34. Neuvonen PJ, Niemi M, Backman JT: Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. *Clin Pharmacol Ther* 80:565-581, 2006

35. Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H, Sugiyama Y: Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* 50:387-412, 1998
36. Fonseca V, Rosenstock J, Patwardhan R, Salzman A: Effect of metformin and rosiglitazone combination therapy in patients with type 2 diabetes mellitus: a randomized controlled trial. *JAMA* 283:1695-1702, 2000
37. Gomez-Perez FJ, Fanghanel-Salmon G, Antonio Barbosa J, Montes-Villarreal J, Berry RA, Warsi G, Gould EM: Efficacy and safety of rosiglitazone plus metformin in Mexicans with type 2 diabetes. *Diabetes Metab Res Rev* 18:127-134, 2002
38. Moses R, Slobodniuk R, Boyages S, Colagiuri S, Kidson W, Carter J, Donnelly T, Moffitt P, Hopkins H: Effect of repaglinide addition to metformin monotherapy on glycemic control in patients with type 2 diabetes. *Diabetes Care* 22:119-124, 1999
39. Di Cicco RA, Allen A, Carr A, Fowles S, Jorkasky DK, Freed MI: Rosiglitazone does not alter the pharmacokinetics of metformin. *J Clin Pharmacol* 40:1280-1285, 2000
40. Grube M, Kock K, Oswald S, Draber K, Meissner K, Eckel L, Bohm M, Felix SB, Vogelgesang S, Jedlitschky G, Siegmund W, Warzok R, Kroemer HK: Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clin Pharmacol Ther* 80:607-620, 2006
41. GLUCOPHAGE GX: metformin hydrochloride extended-release tablets. *Bristol-Myers Squibb Company Princeton, NJ 08543* article online, 2000
42. Hatorp V, Walther KH, Christensen MS, Haug-Pihale G: Single-dose pharmacokinetics of repaglinide in subjects with chronic liver disease. *J Clin Pharmacol* 40:142-152, 2000
43. Kirchheiner J, Thomas S, Bauer S, Tomalik-Scharte D, Hering U, Doroshenko O, Jetter A, Stehle S, Tshauridu M, Meineke I, Brockmoller J, Fuhr U: Pharmacokinetics and pharmacodynamics of rosiglitazone in relation to CYP2C8 genotype. *Clin Pharmacol Ther* 80:657-667, 2006

**TABLE 1.** Comparison of pharmacokinetic data of oral antidiabetic drugs in humans with IC<sub>50</sub> values of OATP1B1-, OATP1B3-, OATP2B1-mediated BSP uptake and OCT1-mediated MPP<sup>+</sup> uptake obtained in the present study.

	dosage [mg]	C <sub>max</sub> [mg/l]	C <sub>max</sub> [μM]	C <sub>port. vn.</sub> [μM]	IC <sub>50</sub> OATP1B1 [μM]	IC <sub>50</sub> OATP1B3 [μM]	IC <sub>50</sub> OATP2B1 [μM]	IC <sub>50</sub> OCT1 [μM]	references
Metformin	1 x 850 <sup>a</sup>	5.9 <sup>a</sup>	35.9	356.7	> 100 (BSP)	> 100 (BSP)	> 100 (BSP)	> 1000 (MPP <sup>+</sup> )	<sup>a</sup> (41)
Repaglinide	1 x 4 <sup>b</sup>	0.1 <sup>b</sup>	0.2	0.8	2.2 (BSP) † (pravastatin)	5.6 (BSP) † (pravastatin)	5.2 (BSP)	1.8 (MPP <sup>+</sup> ) 1.6 (metformin)	<sup>b</sup> (42)
Rosiglitazone	1 x 8 <sup>c</sup>	0.8 <sup>c</sup>	1.7	2.7	6.0 (BSP) ‡ (pravastatin)	10.9 (BSP) ‡ (pravastatin)	5.2 (BSP)	30.4 (MPP <sup>+</sup> ) 6.9 (metformin)	<sup>c</sup> (43)

Note. IC<sub>50</sub> data are derived from the measurements shown in Figures 3 to 8; respective tested substrates are indicated in parenthesis; † = IC<sub>50</sub> value was not determined, but there is an inhibition at all inhibitor concentrations investigated; ‡ = IC<sub>50</sub> value was not determined, but there is an inhibition at the highest inhibitor concentration tested; C<sub>max</sub> = maximal plasma concentration in the systemic circulation, C<sub>port.vn.</sub>= predicted concentration in portal venous blood [according to (35)]

## FIGURE LEGENDS

**FIGURE 1.** Characterization of stably transfected MDCK cells. A, Intracellular MPP<sup>+</sup> accumulation in MDCKII-OCT1 and MDCKII-control (Control) cells after 10 min incubation with MPP<sup>+</sup> (50 μM). B, Net intracellular MPP<sup>+</sup> accumulation in MDCKII-OCT1 cells after 10 min incubation with increasing MPP<sup>+</sup> concentrations. The uptake was obtained by subtracting the uptake in vector-transfected cells (control) from that in OCT1-expressing cells. K<sub>m</sub> and V<sub>max</sub> values were calculated by fitting the data to a one site binding curve. C, Intracellular metformin accumulation in MDCKII-OCT1 and MDCKII-control (Control) cells after 10 min incubation with metformin (10 μM). Each value is the mean value ± standard error (n = 3). \*\*\* P < 0.001 vs control.

**FIGURE 2.** Inhibition of OATP-mediated BSP uptake by oral antidiabetic drugs. Inhibitory effect of repaglinide, rosiglitazone and metformin on OATP1B1-, OATP1B3-, and OATP2B1-mediated BSP uptake after 10 min incubation. IC<sub>50</sub> values were calculated by fitting the data to a sigmoidal dose-response regression curve. Data are shown as the percentage of the mediated BSP uptake in the absence of oral antidiabetics. Each value is the mean value ± standard error (n = 3).

**FIGURE 3.** Inhibition of the OATP1B1- and OATP1B3-mediated pravastatin uptake by repaglinide and rosiglitazone. Inhibitory effect using 10 μM and 100 μM of the oral antidiabetics repaglinide and rosiglitazone on A, OATP1B1- and B, OATP1B3-mediated pravastatin (50 μM) uptake after 10 min incubation. Data are shown as the percentage of the transporter-mediated pravastatin uptake in the absence of oral antidiabetics (Control). Each value is the mean value ± standard error (n = 3). \* P < 0.05; \*\* P < 0.01 vs control.

**FIGURE 4.** Inhibition of MPP<sup>+</sup> and metformin uptake by oral antidiabetics in MDCKII-OCT1 cells. A, Inhibitory effect of repaglinide, rosiglitazone and metformin on OCT1-mediated MPP<sup>+</sup> (30 μM) uptake after 10 min incubation. Data are shown as the percentage of the OCT1-mediated MPP<sup>+</sup> uptake in the absence of repaglinide, rosiglitazone or metformin. B, Inhibitory effect of repaglinide and rosiglitazone on OCT1-mediated metformin (10 μM) uptake after 10 min incubation. Data are shown as the percentage of the OCT1-mediated metformin uptake in the absence of repaglinide or rosiglitazone. IC<sub>50</sub> values were calculated by fitting the data to a sigmoidal dose-response regression curve. Each value is the mean value ± standard error (n = 4).

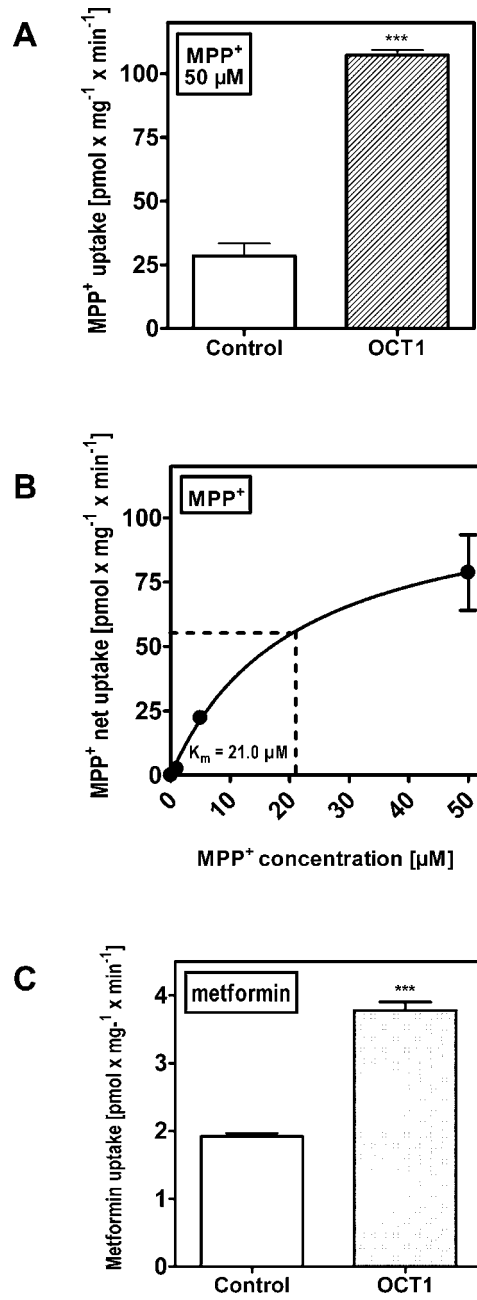


Fig. 1

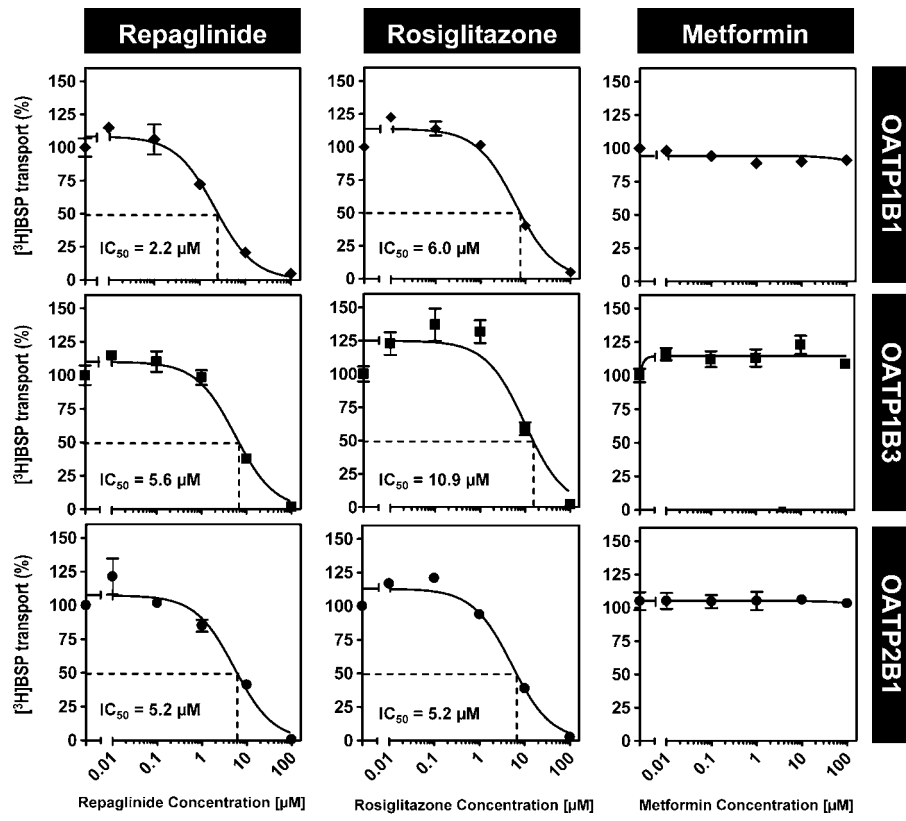


Fig. 2

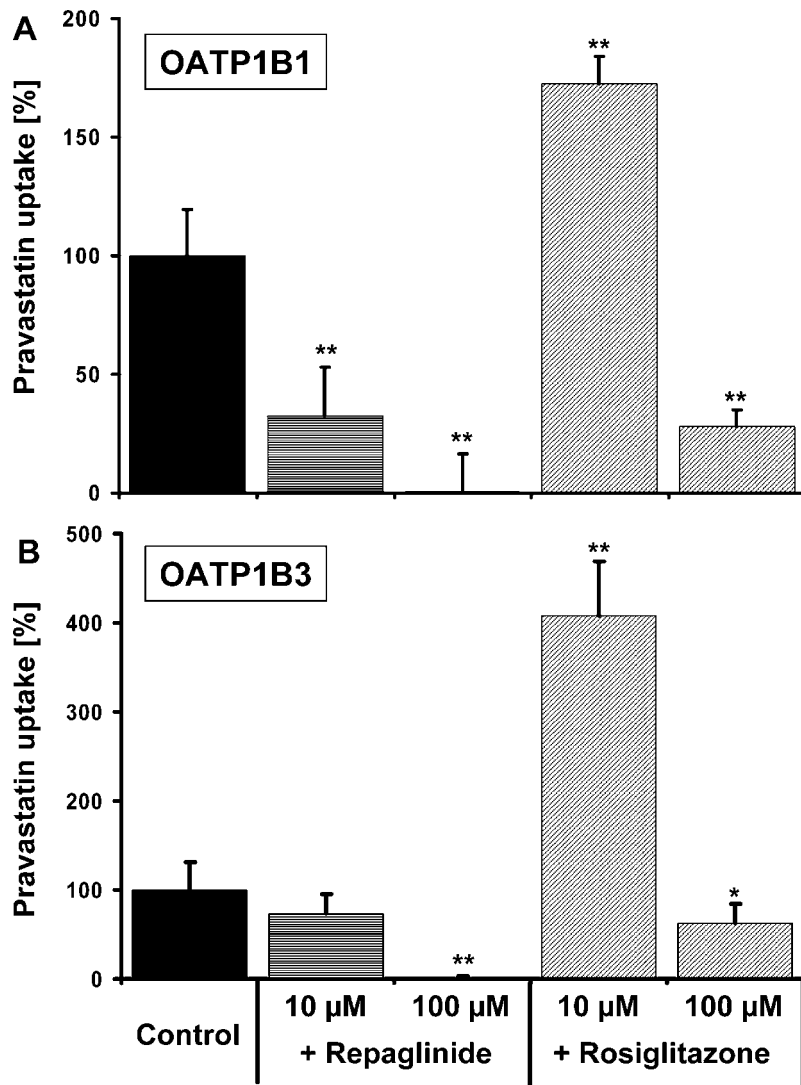


Fig. 3

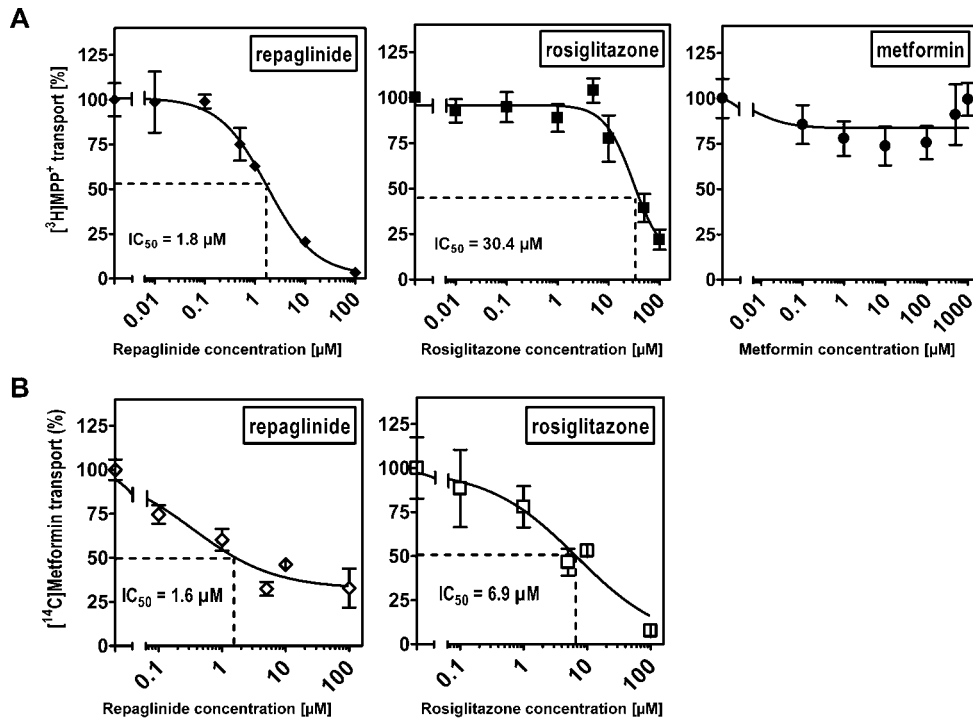


Fig. 4