Rodent Oat1 vs. Human OAT1

Introduction: Rodent organic anion transporter 1 (Oat1) of the Slc22 gene family are polyspecific transporters mainly located in kidneys. Several Oats are expressed also in liver and brain. Mouse (m) Oat1 and rat (r) Oat1 consist of 551 and 545 amino acids, respectively. They posses an amino acids sequence identity of 86% and 87% compared to the homologouse gene of human OAT1. OATs interact with endogenous metabolic end products such as urate and acidic neurotransmitter metabolites, as well as with a multitude of widely used drugs, including antibiotics, antihypertensiva, antivirals, anti-inflammatory drugs, diuretics and uricosurics. Thereby, OATs play an important role in renal and hepatic drug elimination and have an impact on pharmacokinetics. Since OATs are typically found at boundary epithelia, these transporters play an important role in absorption, distribution and excretion of drugs. Moreover, OATs can be the site of drug-drug interactions during competition of two or more drugs for the same transporter and mediate cell damage by transporting cytotoxic compounds. For human OAT1 regulatory agencies (FDA, EMA and Japan) decided that renal eliminated drugs has to be tested for drug-drug interaction, in vitro. Concerning that ADME-Tox related data are initially generated in rodents, it is important to consider species differences *in vivo* as well as *in vitro*. Therefore, PortaCellTec Biosciences GmbH provides comparable cell systems, to detect substrate and inhibitory differences between human and rodent drug transporters, *in vitro.*

Methods: PortaCellTec (PCT) generated HEK293 cell lines stably expressing mouse (m)Oat1, rat (r)Oat1 and human (h) OAT1 transporter proteins and validated each cell-transporter system with a reference substrate (^{3}H-PAH (p-aminohippuric acid)) and different inhibitors. To perform uptake experiments, three days after cell seeding, the uptake was initiated by adding the reference substrate in the absence and presence of an inhibitor. To terminate the uptake cells were washed three times with cold assay buffer. The radio-labelled content (^{3}H) of each cell lysate was analyzed by liquid scintillation counting.

mOat1 – Slc22a6

Substrate	Inhibitor	Kinetic parameters	References
PAH	---	K_m = 32±5 µM	K_m = 37.3 µM (Kuze, 1999) K_m = 162 µM (You, 2000)
PAH	Probenecid	IC_{50} = 12±1 µM	K_i = 6.4 µM (Kaler, 2007)

Figure 1 Concentration dependent mOat1 mediated PAH net-uptake

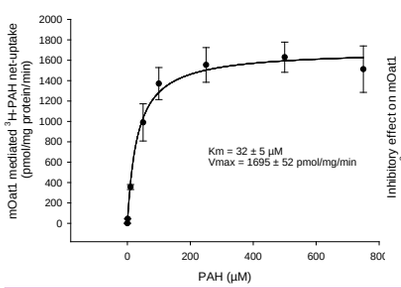
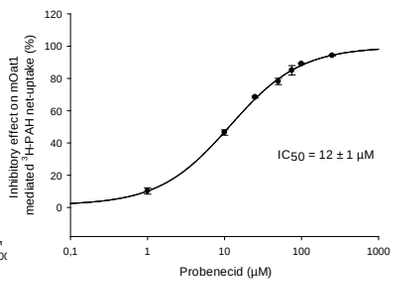


Figure 2 Inhibition of mOat1 mediated PAH net-uptake by probenecid



rOat1 – Slc22a6

Substrate	Inhibitor	Kinetic parameters	References
PAH	---	K_m = 28±8 µM	Km = 31 µM (Uwai, 2000a) Km = 43 µM (Takeda, 1999) Km = 47 µM (Nagata, 2002)
PAH	Probenecid	IC_{50} = 14±8 µM	IC_{50} = 15.3 µM (Khamdang, 2004) IC_{50} = 18.6 µM (Uwai, 2000b)

Figure 3 Concentration dependent rOat1 mediated PAH net-uptake

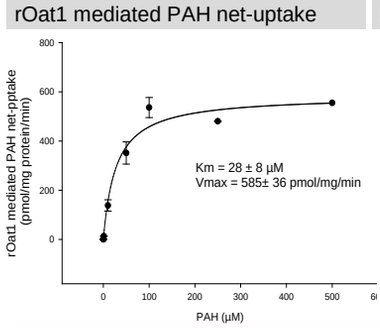
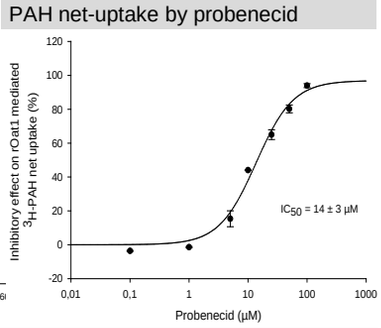


Figure 4 Inhibition of rOat1 mediated PAH net-uptake by probenecid



hOAT1 – SLC22A6

Substrate	Inhibitor	Kinetic parameters	References
PAH	---	K_m = 94±26 µM	K_m = 28 µM (Ueo, 2005) K_m = 113 µM (Zhang, 2008)
PAH	Probenecid	IC_{50} =31±3µM	IC_{50} = 6.5 µM (Ho, 2000)

Figure 5 Concentration dependent hOAT1 mediated PAH net-uptake

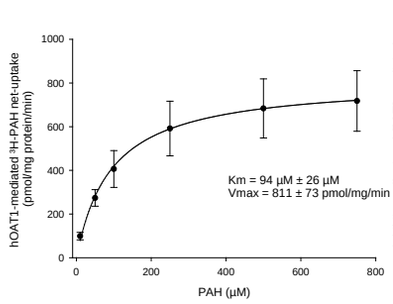
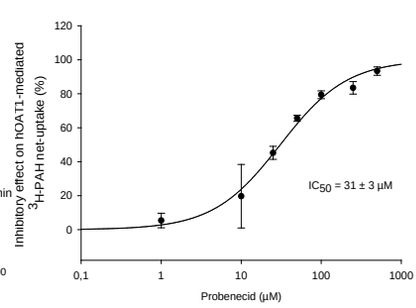
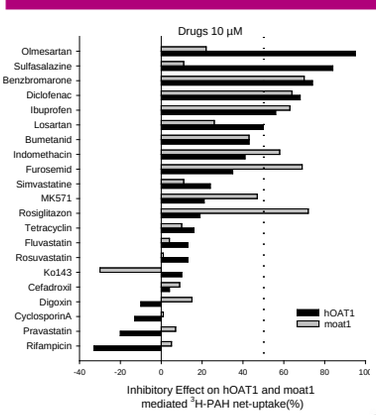
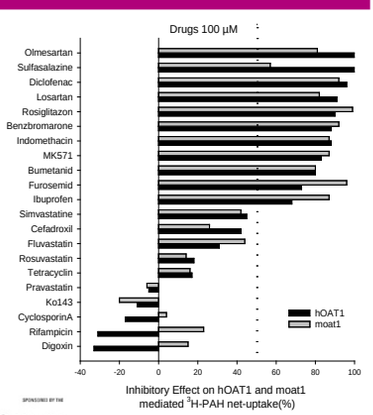


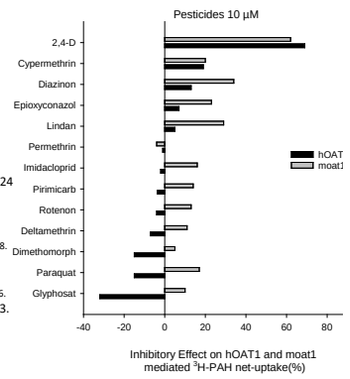
Figure 6 Inhibition of hOAT1 mediated PAH net-uptake by probenecid

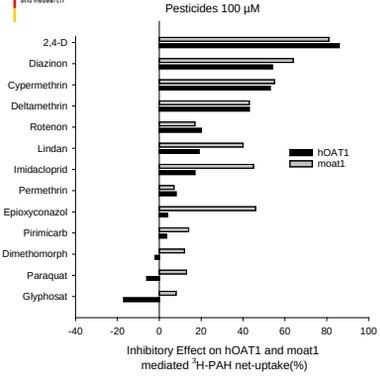


hOAT1 vs. moat1









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Rodent Oat3 vs. Human OAT3

Introduction: Rodent organic anion transporter 3 (Oat3) of the Slc22 gene family are polyspecific transporters mainly located in kidneys. Several Oats are expressed also in liver and brain. Mouse (m) Oat3 and rat (r) Oat3 consist of 536 and 537 amino acids, respectively. They posses an amino acids sequence identity of 79% and 78% compared to the homologous gene of human hOAT3. OATs interact with endogenous metabolic end products such as urate and acidic neurotransmitter metabolites, as well as with a multitude of widely used drugs, including antibiotics, antihypertensiva, antivirals, anti-inflammatory drugs, diuretics and uricosurics. Thereby, OATs play an important role in renal and hepatic drug elimination and have an impact on pharmacokinetics. Since OATs are typically found at boundary epithelia, these transporters play an important role in absorption, distribution and excretion of drugs. Moreover, OATs can be the site of drug-drug interactions during competition of two or more drugs for the same transporter and mediate cell damage by transporting cytotoxic compounds. For human hOAT3 regulatory agencies (FDA, EMA and Japan) decided that renal eliminated drugs has to be tested for drug-drug interaction, in vitro. Concerning that ADME-Tox related data are initially generated in rodents, it is important to consider species differences *in vivo* as well as *in vitro*. Therefore, PortaCellTec Biosciences GmbH provides comparable cell systems, to detect substrate and inhibitory differences between human and rodent drug transporters, *in vitro.*

Methods: PortaCellTec (PCT) generated HEK293 cell lines stably expressing mouse (m)Oat3, rat (r)Oat3 and human (h)OAT3 transporter proteins and validated each cell-transporter system with a reference substrate (^{3}H-estrone-sulfate (ES)) and different inhibitors. To perform uptake experiments, three days after cell seeding, the uptake was initiated by adding the reference substrate in the absence and presence of an inhibitor. To terminate the uptake cells were washed three times with cold assay buffer. The radio-labelled content (^{3}H) of each cell lysate was analyzed by liquid scintillation counting.

mOat3 – Slc22a8

Substrate	Inhibitor	Kinetic parameters	References
Estrone-sulfate	---	K_m = 70±19 µM	K_m = 12.2 µM (VanWert, 2008)
Estrone-sulfate	Probenecid	IC_{50} = 6.5±1.6 µM	K_i = 4.6 µM (Eraly, 2008)

Figure 3 Concentration dependent mOat3 mediated ES net-uptake

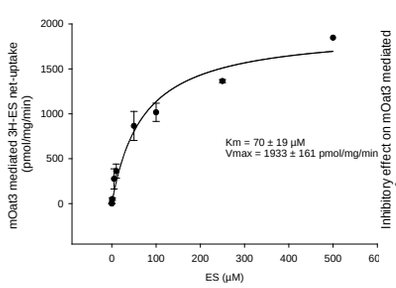
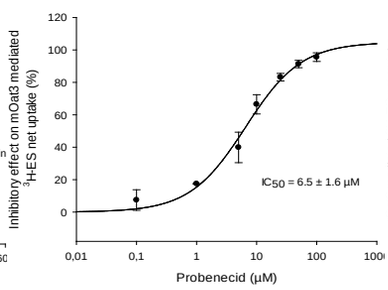


Figure 4 Inhibition of mOat3 mediated ES net uptake by Probenecid



rOat3 – Slc22a8

Substrate	Inhibitor	Kinetic parameters	References
Estrone-sulfate	---	K_m = 38±11 µM	K_m = 7.1 µM (Minematsu, 2008) K_m = 34 µM (Hasegawa, 2003)
Estrone-sulfate	Probenecid	IC_{50} = 13±4 µM	IC50 =6.0 µM (Khamdang, 2004)

Figure 7 Concentration dependent rOat3 mediated ES net-uptake

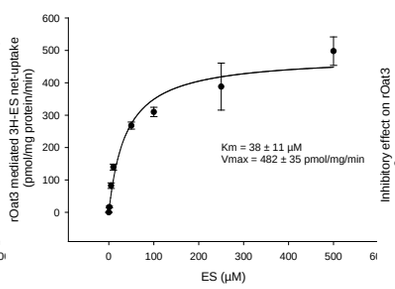
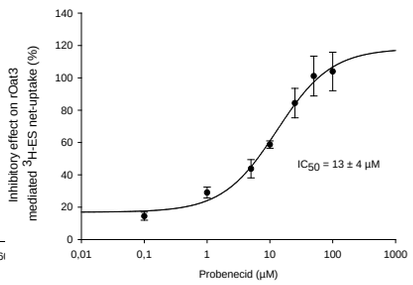


Figure 8 Inhibition of rOat3 mediated ES net uptake by Probenecid



hOAT3 – SLC22A8

Substrate	Inhibitor	Kinetic parameters	References
Estrone-sulfate	---	K_m = 20±5 µM	K_m = 6.3 µM (Ueo, 2005)
Estrone-sulfate	Probenecid	IC_{50} = 23±2µM	IC_{50} = 4.7 µM (Srimaroeng 2005)

Figure 5 Concentration dependent hOAT3 mediated ES net-uptake

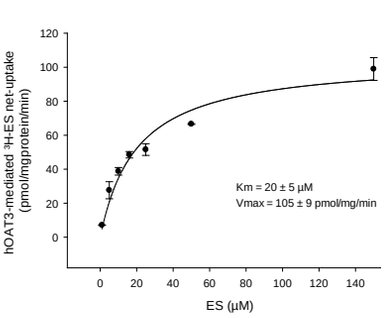
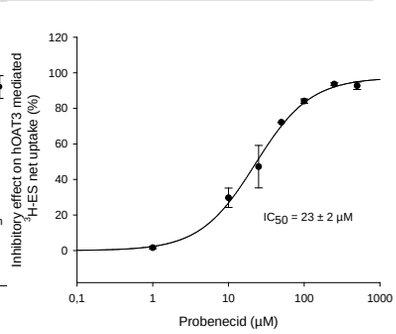
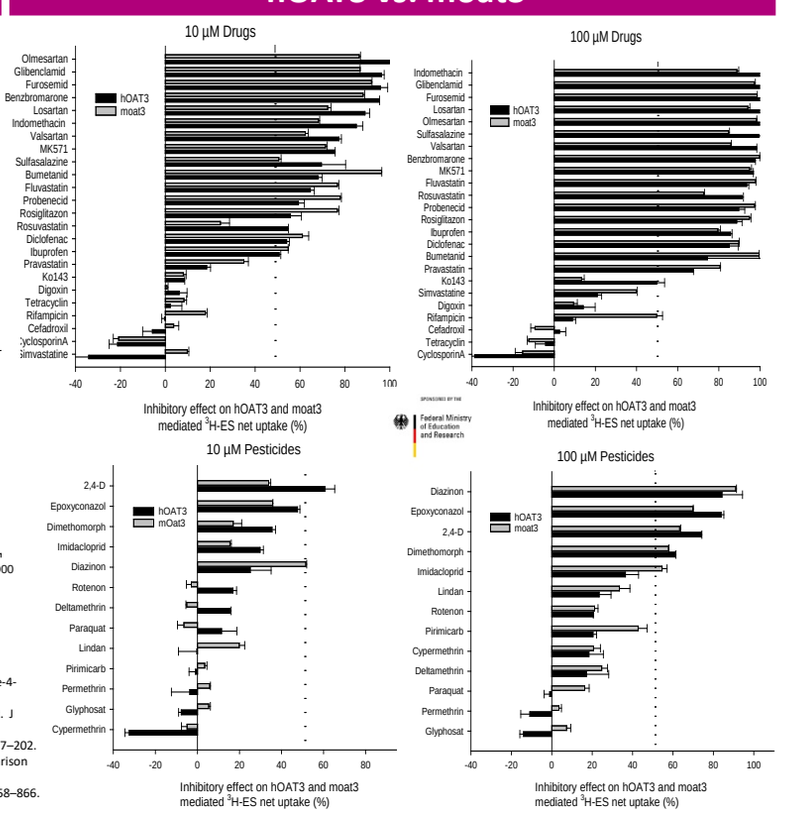


Figure 6 Inhibition of hOAT3 mediated ES net uptake by Probenecid



hOAT3 vs. moat3



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