Transporters as mediators of cisplatin effects and side-effects

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The use of the effective antineoplastic agent cisplatin is hampered by serious side effects, such as nephro-, oto-, and neurotoxicity. In this work, we studied whether organic cation transporters (OCT) mediate the uptake and hence the toxicities of cisplatin *in vitro* and *in vivo*. Interaction of cisplatin with the transport of the fluorescent cation 4-(4-(dimethylamino)styril)-methylpyridinium (ASP^+) was investigated in HEK293 cells stably transfected with hOCT1 or hOCT2 and in freshly isolated human hepatocyte and human proximal tubules. Cisplatin preferentially inhibited ASP^+ transport via hOCT2. Incubation of hOCT2 cells with cisplatin induced apoptosis, which was completely suppressed by co-incubation by the hOCT2 substrate cimetidine. *In vivo*, the effects of cisplatin treatment on kidney and hearing (auditory brainstem response, ABR) functions were compared in wild type (WT) and OCT1/2 double knock-out (KO) mice. While in WT cisplatin led to reduced ABR and increased renal glucose, water, protein excretion and apoptosis, no sign of ototoxicity and only mild nephrotoxicity was observed after cisplatin treatment of KO mice. Co-medication of WT mice with cisplatin and cimetidine protected from ototoxicity and partly from nephrotoxicity. We also showed that OCT2 is expressed on hair cells of the cochlea and in dorsal root ganglia. Tumour-derived cell lines did not show a significant expression of mRNA for OCT2, suggesting that cisplatin uptake is mediated by other mechanisms here. These findings are very important for establishing chemotherapeutical protocols aimed at maximizing the antineoplastic effect of cisplatin, while reducing the risk of toxicities. Supported by IZKF Münster, Grant Cia2/013/13.

KEY WORDS: *cisplatin; cochlea; Corti-organ; dorsal root ganglia; kidneys; proximal tubules; side effects; transporters; tumour cells*

CFEX (Slc26a6) in rat kidneys, liver, and small intestine in an experimental model of oxalate nephrolitiasis

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CFEX (chloride/formate exchanger; Slc26a6) is an important anion exchanger of chloride, bicarbonate, oxalate (OX), formate, and hydroxyl ions in kidneys, liver, and the small intestine. Studies on *CFEX*-knockout mice indicated a possible role of CFEX in the development of hyperoxaluria and OX urolithiasis/nephrolithiasis, which in humans is more frequent in men. Here we studied the expression of the CFEX protein and mRNA in the organs of male and female rats, and employed a rat model of ethylene glycol (EG)-induced OX urolithiasis in order to correlate the expression of CFEX with sex-related hyperoxaluria. Rats drank EG in water (0.75 % vol/vol) or water (control) for 30 days. Tissue expressions of the CFEX protein and mRNA were analysed by immunochemical methods and qRT-PCR, respectively. The specificity of an anti-CFEX antibody, used in immunochemical studies, was confirmed in HEK293 cells transiently transfected with *CFEX* cDNA. In kidneys, the CFEX protein was immunolocalized to the proximal tubule brush-border membrane (BBM) with segmental (S3>>S1~S2) and sex (male>female) differences. Sex-unrelated expression was detected in the BBM of enterocytes (duodenum>jejunum) and in the hepatocyte canalicular membrane. In immunoblots, the CFEX protein band of ~120 kDa in various organs showed an expression pattern comparable to that in immunocytochemistry; however, renal *CFEX* mRNA expression was not sex-dependent. Compared to controls and EG-treated females, the EG-treated male rats exhibited hyperoxalemia, hyperoxaluria and OX crystaluria, but the expression of *CFEX* mRNA and protein remained unaffected in the organs of both sexes. Thus, basic CFEX expression in both rat sexes was sufficient for OX handling even upon EG-treatment, indicating that in rats, CFEX plays no major role in generating EG-induced hyperoxaluria and nephrolithiasis.

KEY WORDS: *ethylene glycol; hyperoxaluria; immunocytochemistry; transporters; proximal tubule; qRT-PCR; urolithiasis; Western blotting*

Inhibitors of glucose transporter SGLT1 in the treatment of *diabetes mellitus* will not act only in the kidneys; the transporter is also present in other rodent and human organs

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Diabetes mellitus, one of the most common chronic diseases in population, is becoming a major health and economic problem. Current therapies with insulin and metformin, aimed at reducing blood glucose, are often ineffective and/or problematic due to the induction of hypoglycemia, body weight gain, and occasional death resulting from cardiovascular disease. A novel generation of oral anti-diabetics inhibits sodium-D-glucose cotransporters in the small intestine (SGLT1/*SLC5A1*), thus diminishing the absorption of glucose from the diet, as well as in the kidneys (SGLT1 and/or SGLT2/*SLC5A2*), thus decreasing glucose reabsorption along the nephron and enhancing its excretion through urine. Overall, this improves glycemia, reduces body weight, lowers blood pressure, and decreases damage to the cardiovascular system. However, our recent studies showed that in humans, SGLT1 is not expressed only in the intestinal and renal epithelium; it was also detected in the liver (bile duct epithelium), lungs (bronchiolar Clara cells and alveolar type II cells), and heart (blood capillaries). These places represent possible targets for novel SGLT1 inhibitors. SGLT1 or dual (SGLT1+SGLT2) inhibitors may inhibit various SGLTs-related functions, such as fluid absorption in the lungs, energy supply to Clara cells, and glucose release from the heart capillaries, and may thus cause functional disorders. In addition, our novel unpublished data showed that in mice, Sglt1 is localized in the kidneys, small intestine, liver, pancreas, salivary glands, tongue, prostate, seminal vesicles, and uterus. The newly discovered localizations of SGLT1/Sglt1 suggest certain novel functions for this transporter, which could be of great physiological and biomedical importance.

KEY WORDS**:** *human organs; immunocytochemistry; mouse organs; qRT-PCR; sodium-D-glucose cotransporters; SGLT1; SGLT2*

Genetic variability in organic cation transporters: pharmacology, pathophysiology, and beyond

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Naturally occurring genetic variants substantially affect the expression and function of organic cation transporters. Therefore, these variants may lead to inter-individual variations in plasma and organ concentrations of endogenous molecules and drugs and may cause variations in drug pharmacokinetics, efficacy and toxicity, or may confer susceptibility to diseases. Common genetic variants causing loss of OCT1 activity have been shown to affect the hepatic uptake and pharmacokinetics of the drugs morphine, tramadol and tropisetron. On the other hand, genetic variants causing loss of OCT1 activity are suggested to modulate the efficacy of drugs acting in the liver, like metformin. Here we give an overview on the available data about the genetically-determined loss of OCT1 activity and discuss potential applications for personalised drug therapy. Furthermore, specific global patterns of loss of OCT1 activity were observed. We discuss how these patterns may confer inter-ethnical variability of pharmacokinetics and efficacy of clinically relevant drugs and how they may point to selection pressure for losing or maintaining OCT1 activity. In OCT2, the common polymorphism Ala270Ser was repeatedly reported to associate with inter-individual variability in metformin pharmacokinetics. We present meta-analyses of currently available studies showing the inconsistency and limited size of these effects. In contrast, polymorphisms in OCTN1 and OCTN2 were repeatedly reported as risk factors for Crohn's disease. Meta-analyses of available studies are presented illustrating the high consistency of these observations. Finally we discuss the potential effects of regulatory polymorphisms in the MATE1 and MATE2K genes on the pharmacokinetics and efficacy of metformin.

KEY WORDS**:** *Crohn's disease; genetic polymorphisms; hepatic uptake; metformin; morphine; pharmacokinetics; renal clearance; SNPs; tramadol; tropisetron*

Importance of transporters in the prediction of drug efficacy and adverse events

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An important role of drug transporters in modulating pharmacokinetic and pharmacodynamic properties has been documented for many drugs. Many studies have investigated the association between gene polymorphisms of the efflux transporters belonging to the ATP-binding cassette (ABC) superfamily of membrane proteins, as well as of influx transporters of the SLC superfamily, and therapy response and clinical outcomes. Convincing results have been obtained for ABCB1 gene variants and variability of therapy by digoxin, HIV protease inhibitors, some antiepileptics, antidepressants, antipsychotics, immunosuppressants; ABCC2 and mycophenolic acid, anticancer drugs like methotrexate, cisplatin, irinotecan, antibiotics; ABCG2 and anticancer drugs, statins, alopurinol, cimetidin, and lamotrigin. Drug-drug interactions on the level of variable drug metabolism by phase I and phase II enzymes and drug transporters often called for a phase III modulate at many barrier tissues – such as the intestine, liver, blood-brain barrier, kidney, placenta – plasma and cerebrospinal fluid drug concentrations that can lead to non-responsiveness, resistance or serious or even lethal adverse drug reactions. The most prominent case is the development of rhabdomyolysis due to statin therapy in case of variant/ineffective allele carriers of SLCO1B1 521C>T with an aggravating role of ABCG2 421C>A and some CYP450 gene polymorphisms. Data also emphasize the role of ABCB1 and ABCG2 polymorphisms as promising predictors of the clinical outcome of tyrosine kinase inhibitor (TKI) therapy with drugs like imatinib, nilotinib, and sunitinib. Based on clinical evidence of drug transporter-mediated drug-drug interactions (DDIs) and some functional polymorphisms affecting drug efficacy and safety, both the US Food and Drug Administration and European Medicines Agency recommend preclinical evaluation and, when appropriate, clinical assessment of transporter-mediated DDIs. Although variability of bioavailability and drug response may be attributed to certain polymorphisms in transporter genes, transcriptional regulation or post-transcriptional modification in certain cases seems to be even more critical.

KEY WORDS: *adverse drug reactions; drug bioavailability; drug-drug interaction; drug transporters; genetic polymorphism*

Age and sex differences in expression of P-glycoprotein (P-gp/Mdr1/Abcb1) in rat liver and kidneys

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P-glycoprotein (P-gp; ABCB1 in humans/Abcb1 in rodents) is an ATP-dependent multidrug efflux transporter in the cell membrane, also known as the multidrug resistant protein 1 (MDR1 in humans/Mdr1 in animals), constitutively expressed in the a) bile canaliculi of hepatocytes, b) brush-border membrane of the renal proximal tubule cells, c) luminal membrane of the intestinal enterocytes, d) blood-brain, blood-testis, and mother-foetus barriers, and e) hematopoietic cells. P-gp mediates the transport of some endogenous compounds and various xenobiotics (drugs, toxins, environmental organic compounds, and their metabolites) out of the cells, thus protecting the cell's interior from the potentially toxic effects of these compounds. Although P-gp is a highly studied transporter in a variety of (patho)physiological conditions in human and animal organs, its age-dependent expression is still a controversial issue. Here, we investigated the ontogenic pattern of P-gp protein expression in rat liver and kidneys to provide insight into the drug transport capacity in these organs at different ages. P-gp protein expression was studied by immunocytochemistry and Western blotting in organs from neonatal (age, 1 day), prepubertal (age, 3 weeks), adult (age, 3 months), and old (age, 2 years) male and female Wistar rats. In both sexes, the liver P-gp expression pattern was: neonatal (highest) > prepubertal > adult < old. In the kidneys, the P-gp expression exhibited the pattern: neonatal < prepubertal < adult (highest) > old. Better knowledge of the ontogeny of P-gp expression may improve experimental design and the interpretation of results of toxicity studies in juvenile animals as well as the understanding of drug toxicity in different age groups in translational studies.

KEY WORDS: *age differences; immunocytochemistry; kidney; liver; Mdr1; proximal tubule; P-gp; Western blotting*

Identification of xenobiotic interactors with multidrug and toxin extrusion (MATE/ SLC47) proteins in zebrafish (*Danio rerio*)

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Multidrug and toxin extrusion (MATE) proteins are involved in the extrusion of endo- and xenobiotics across the plasma membrane, similar to the ATP-binding cassette (ABC) transporters but without the consumption of ATP. MATEs are conserved from bacteria to mammals with a differing number of genes within groups. In humans, three MATEs have been found (MATE1, 2, and 2k), whereas in zebrafish (*Danio rerio)*, we have found six members annotated as DrMate 3-8 which form a distinct cluster separated from the tetrapod MATEs. Tissue expression profiling showed a high expression of zebrafish MATEs in toxicologically important tissues, kidney and liver, as well as in the testes. MATEs transport activity was analysed in transiently overexpressing HEK293 cell system measuring the uptake of model cationic fluorescent dyes. Assay conditions were optimised so that the protein interaction with a battery of toxicologically interesting endo- and xeno-biotics could be evaluated. Basic kinetic parameters together with the type of interaction – whether a compound is transported or if it merely inhibits the transport activity – were determined for over 20 selected physiological and/or xenobiotic interactors of mammalian MATEs, including hormones, bile salts, drugs, and pesticides.

KEY WORDS**:** *efflux transporters; MATEs; physiological and xenobiotic interactors; zebrafish*

Functional and structural characterization of organic cation transporter 1 (Oct1) in zebrafish (*Danio rerio*)

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Organic cation transporters (OCTs) are members of the SLC22 family within the SLC (Solute Carriers) superfamily of membrane proteins. These transporters are responsible for the uptake of numerous organic cations and neutral molecules, alongside other inorganic ions. There are three OCTs in humans, two of which play crucial roles in ADME (administration, distribution, metabolism and excretion) processes, with high expression of OCT1 in liver and OCT2 in kidneys. In zebrafish, an important vertebrate model organism, there are two Oct members, with Oct1 dominantly expressed in kidneys and livers, where it potentially has the compensatory role of human OCT1 and OCT2, whereas Oct2 showed lower expression in toxicologically less relevant tissues, which indicates its potentially more specific physiological role. Using transiently transfected human embryonic kidney cells (HEK239) as a heterologous expression system, we developed an *in vitro* tool for the functional analysis of Oct1 by measuring the uptake of five identified fluorescent substrates of Oct1. Functional analysis revealed the interaction of Oct1 with numerous endo- and xenobiotics. Steroid hormones showed potent inhibition of 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP+) uptake by Oct1, with K_i values in low micromolar range, along with potent interactions with numerous xenobiotics ranging from various pharmaceuticals to deleterious environmental contaminants such as organotin compounds. However, further analysis focused on the type of interaction with the identified interactors was limited due to the complexity of the Oct1 active site, which we characterized in more detail using homology modelling and molecular docking analysis. The structural models of zebrafish Oct1 and human OCT1 and OCT2 revealed a characteristic transmembrane organization of proteins and spacious active regions with several binding sites. Our research offers novel insight into the function and transport mechanism of Oct1 as a potentially crucial factor in steroid hormone homeostasis and toxicological response in zebrafish.

KEY WORDS**:** *functional characterization; organic cation transporters; physiological and xenobiotic interactors; zebrafish*

Interactions of secondary metabolites from cyanobacteria and invasive tropical algae with phase 0 membrane transporters in zebrafish (*Danio rerio*)

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One advantageous feature of cyanobacteria and invasive tropical algae from the genus *Caulerpa* is their ability to produce secondary metabolites with diverse biological activities. Some secondary metabolites are toxic and thus represent a significant threat to the environment and animal/human health, especially during periods of intensive blooms. So far, the understanding of their interaction with membrane transporters involved in the cellular defence mechanism has been poor. Therefore, the main goal our study was to determine the interactions of secondary metabolites from *C. racemosa*, *C. taxifolia* and selected cyanobacterial strains, including genera *Anabaena*, *Nostoc*, *Phormidium* and *Oscillatoria*, with the phase 0 membrane transporters involved in the cellular detoxification mechanism of an important model vertebrate species, the zebrafish (*Danio rerio*). The toxic inhibitory effects of secondary metabolites from *Caulerpa* and cyanobacterial strains to the activity of zebrafish anion (DrOatp1d1) and cation (DrOct1) uptake transporter were determined using the human embryonic kidney (HEK293) expression system. In addition, we performed a preliminary identification of the biologically active substances that cause the observed toxic effects using the effects-directed analyses (EDA) approach. Significant toxicity for these complex biological samples towards toxicologically relevant zebrafish uptake transporters DrOatp1d1 and DrOct1 was determined. Caulerpin (CLP) was determined as the major metabolite in *C. racemosa* while caulerpenyne (CYN) appeared to be the dominant biologically active compound in *C. taxifolia*. CYN was confirmed as an inhibitor of the DrOatp1d1 anion transporter. Finally, aquatic cyanobacterial strains, especially the *Oscillatoria* strain, showed the most significant and potentially (eco)toxicologically highly relevant inhibitory effects towards the transport activity of DrOatp1d1 and DrOct1 transporters.

KEY WORDS**:** *cellular detoxification; cyanobacteria; invasive tropical algae; phase 0 membrane transporters; secondary metabolites; zebrafish (*Danio rerio*)*

Invasive *vs.* native bivalves - differences in tolerance to anthropogenic stress

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Tolerance towards environmental stress has been frequently considered as one of the key determinants of invasion success. However, empirical evidence supporting the assumption that invasive species endure unfavourable conditions better compared to native species is limited and has even yielded opposing results. We examined tolerance to thermal stress and heavy metal zinc pollution ($ZnCl_2$) in two phylogenetically related and functionally similar freshwater bivalve species; the native *Anodonta anatina* and the invasive *Sinanodonta woodiana*. We assessed their response to stress using several cellular response assays: metabolic rates (ETS - electron transport system), efficiency of the multixenobiotic resistance (MXR) mechanism activity, and enzymatic biomarkers (ChE - cholinesterase, GST - glutathione-S-transferase and CAT - catalase). Overall, *S. woodiana* coped with unfavourable conditions much better. This was evident from (i) a significantly more pronounced MXR mechanism activity; (ii) significantly higher ETS activity, and (iii) lower response of stress-related enzymes (ChE, GST and CAT) under thermal stress and $ZnCl_2$ pollution. The overall better tolerance to thermal extremes is an especially important physiological advantage for the future of invasion success of *S. woodiana* in European freshwaters, especially in the context of climate change.

KEY WORDS**:** *freshwater mussels; heavy metal zinc pollution; invasion success; metabolic rate; MXR mechanism activity; thermal stress*