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Drug uptake systems in liver and kidney: historic perspective

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Abstract

Drugs and their metabolites are mainly eliminated by excretion into urine and bile. Studies in whole animals, isolated organs, cells and membrane vesicles led to the conclusion that different transport systems are responsible for the transport of different classes of organic compounds (small large, anionic, cationic). In the early 1990's functional expression cloning resulted in the identification of the first transporters for organic anions and cations. Eventually, all the major transport systems involved in the uptake of these organic compounds have been cloned and characterized and we know that they belong to the OATs and OCTs of the *SLC22A* and the OATPs of the *SLCO* superfamilies of polyspecific drug transporters. Today we can explain at the molecular level why small and hydrophilic organic compounds are predominantly excreted via urine while large and amphipathic compounds are mainly excreted via bile and we can start to predict drug-drug interactions for new compounds.

Keywords

Drug transport; OATP; OAT; OCT; liver; kidney

Introduction

Uptake studies to elucidate the mechanisms by which drugs are taken up into the kidney and the liver have been performed for a long time. Investigators have used different experimental setups including perfused organs, isolated cells, isolated membrane vesicles and cloned transporters that are expressed in recombinant systems to characterize these mechanisms. The aim of this article is to summarize some historic milestones that lead to the identification of the individual transporters and to outline the current knowledge with respect to the well characterized drug uptake transporters of the solute carrier families *SLC22A* and *SLCO* that are expressed in the kidney and the liver (1-5). The transporters that are responsible for drug elimination across the brush border membrane in the proximal tubule or across the canalicular membrane of hepatocytes mainly belong to the family of ATP-binding cassette (ABC) transporters and will not be discussed here (for reviews see the special issue "20 years of ABC transporters" Pflügers Arch. Volume 453, Number 5 / February, 2007).

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Conflict of interest:

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Experiments with perfused organs, organ slices and isolated cells

Organic anions

Using the perfused kidneys, isolated tubules and kidney slices, researchers found more than 50 years ago that the kidney has different excretory transport mechanisms for the elimination of organic anions and organic cations. Based on inhibition experiments it could be shown that there is a transport “mechanism” for organic acids and another one for organic bases (6). Excretion of organic anions like penicillin, p-aminohippurate (PAH), and phenol red could be inhibited by probenecid and by each other (7). Transport of cationic compounds like tertaethylammonium and N-methylnicotinamide on the other hand was inhibited by cyanine 863 but was not affected by probenecid or any of the above organic anions indicating different transport systems for organic anions and cations (6). At about the same time it was also found that there was competition between hepatic uptake of bilirubin, bromosulphothalein (BSP) and indocyanine green, indicating a common transport system for these three compounds (8). Kinetic analyses demonstrated mutual competitive inhibition between bilirubin, BSP and indocyanine green, supporting the concept of a common transport mechanism for these three organic anions (9).

But what was the reason that certain organic anions were preferentially eliminated via the kidneys while others were excreted via the liver? Several studies demonstrated that small compounds like phenacetyl glycine (MW 193) and hippuric acid (MW 179) were mainly excreted via the kidneys while larger molecules like bromocresol green (MW 698) and indocyanine green (MW 775) were mainly eliminated via the liver. Tartrazine with an intermediate molecular weight (MW 493) was excreted via both, the kidneys and the liver (10). Based on such studies, it was concluded that the molecular weight seemed to influence whether a molecule was excreted via the kidneys or the liver. To investigate this more systematically, Hirom and colleagues (10) performed an extensive study in rats to determine the fate of 30 aromatic compounds of molecular weights between 100 and 850. They collected urine or bile in control rats and in rats with ligated bile ducts or ligated ureters. Their main finding was that there were three groups of chemicals: Group 1 had molecular weights of less than 350 and was eliminated mainly via urine; even if urinary excretion was prevented, biliary excretion was minimal. Group 2 had molecular weights between 450 and 850 and was excreted predominantly via bile; even if the bile duct was ligated, urinary excretion was minimal. Group 3 had molecular weights between 350 and 450 and was eliminated extensively in both, urine and bile; when one route was blocked, excretion by the other route increased. Thus the authors concluded that urinary excretion was greatest for compounds of the lowest molecular weight and tended to decrease with an increase in molecular weight. Biliary excretion on the other hand increased with increasing molecular weights.

With respect to the mechanism of transport and the driving force, results from experiments performed in kidney slices or isolated tubules were not conclusive. Although it could be shown that a sodium gradient was important for probenecid sensitive PAH uptake (11), and that an exchange mechanism was involved in PAH uptake (12), the exact mechanism could not be resolved until isolated renal basolateral membrane vesicles were used.

In the liver, uptake of bilirubin, BSP and bile acids was studied using the perfused liver and isolated hepatocytes and it could be demonstrated that BSP uptake was saturable, could be inhibited by bilirubin and was dependent on extracellular chloride (13). With respect to bile acids, a sodium-dependent and a sodium-independent transport system was demonstrated (14). Based on studies where uptake of bile acids was inhibited by different xenobiotics, including steroid analogues, cholecystographic agents, and cyclic peptides, a multispecific bile acid transporter that would be able to transport a wide variety of structurally unrelated xenobiotics was proposed (14). Using photo-affinity labeling, candidate proteins of 48-50 kDa

were identified as potential sodium-dependent and of 52-54 kDa as potential sodium-independent bile acid transporters. In addition, a 55 kDa protein was proposed as BSP transporter (15).

Organic cations

Studies to determine the molecular weight threshold for biliary excretion of organic cations were performed for monoquaternary organic cations like tetraethylammonium bromide (16) and diquaternary organic cations like decamethonium bromide (17). It turned out that for monoquaternary organic cations the molecular weight threshold for significant (> 10 % of dose) biliary excretion was about 200 ± 50 (16) while that for diquaternary organic cations was in the region of 500 – 600 (17). However, additional studies with organic anions and cations revealed that a proper balance of polar and non-polar parts in a molecule (amphipathic molecules) was crucial for biliary elimination (18).

Based on all these studies the rule of thumb was established that small and hydrophilic organic compounds were preferentially excreted via the kidneys while large and amphipathic organic compounds were preferentially excreted via the liver.

Experiments using isolated membrane vesicles

Organic anions

Although experiments with whole organs and organ slices were very valuable, it was not possible to separate and characterize the contribution of the different transport systems expressed in the apical or basolateral membrane. Methods were developed to isolate brush-border (19) or basolateral (20) membrane vesicles from renal proximal tubules and basolateral or canalicular membrane vesicles from hepatocytes (21). With these membrane vesicles it became possible to accurately control the buffer composition on both sides of the vesicle membrane and to establish ion gradients across these membranes. Functional studies performed with membrane vesicles identified distinct transport systems for the different classes of organic compounds in the basolateral and the apical membrane of proximal tubular cells as well as in hepatocytes.

It could be demonstrated that the sodium-dependent probenecid sensitive PAH system in the basolateral membrane of the proximal tubule actually consisted of two different transport systems. The sodium-dicarboxylate cotransporter mediated uptake of dicarboxylates like glutarate or α -ketoglutarate into vesicles and generated an in-to-out gradient of the dicarboxylate which was driving the exchange of PAH from out to in (Figure 1) (22). Probenecid, which inhibited PAH uptake, did not affect the sodium dependent dicarboxylate uptake system but inhibited the PAH-dicarboxylate exchanger. Using isolated brush-border membrane vesicles, an electroneutral, pH-gradient driven PAH uptake system that also transported urate, succinate, and lactate was characterized at the luminal side (Figure 1) (22). It was suggested that this system could be responsible for urate reabsorption and would work as an exchanger with PAH that was transported into the cell across the basolateral membrane via the PAH-dicarboxylate exchanger.

In the liver, the sodium-dependent and –independent bile acid uptake systems were further characterized. It turned out that the sodium-dependent system preferentially transported conjugated trihydroxylated bile acids, was electrogenic and based on cis-inhibition studies was multispecific (15). The sodium-independent bile acid system preferentially transported unconjugated bile acids like cholate and could be trans-stimulated by PAH and α -ketoglutarate. Furthermore, sodium-dependent and sodium-independent dicarboxylate transport systems were identified in the basolateral membrane of hepatocytes. Given that α -ketoglutarate uptake was also inhibited by cholate, the presence of a transport system similar to the one in the

basolateral membrane of the proximal tubule, that could couple sodium-dependent dicarboxylate uptake to dicarboxylate organic anion exchange, was proposed for bile acids (15) (Figure 2). Based mainly on cis-inhibition studies it was suggested that the sodium-independent bile acid transporter had a broad substrate specificity and mediated the uptake of bile acids, BSP, cardiac glycosides like ouabain, neutral steroids, linear and cyclic peptides and numerous drugs including statins into hepatocytes (15) (Figure 2). However, the molecular identity of this uptake system could not be resolved with studies using membrane vesicles.

Organic cations

Similar studies with isolated basolateral and brush border membrane vesicles were performed to elucidate transport mechanisms for organic cations. It was demonstrated that uptake across the basolateral membrane occurred via an electrogenic transporter for which the transmembrane electrical potential was the driving force (22). Transport of cations across the brush-border membrane was mediated by two mechanisms; a multispecific, electroneutral cation-proton exchanger and an ATP-dependent multidrug transporter (MDR) that could export cations like verapamil, quinine, and quinidine (22). Together with the electrogenic uptake system at the basolateral membrane, these two transporters were responsible for the renal secretion of cations. For reabsorption of cations like e.g. choline, two systems have been characterized: a low affinity electroneutral system that could be identical to the cation proton antiporter described above and a high affinity electrogenic system that seemed to play an important role in choline reabsorption (23) (Figure 3).

In the liver, Moseley and colleagues characterized an electroneutral N-methylnicotinamide (NMN)/proton exchanger that was not affected by choline, but was inhibited by numerous exogenous and endogenous organic cations including acetylcholine, dopamine, histamine, imipramine, putrescine, spermine, spermidine, and tetraethylammonium (TEA) to varying degrees (24). They later also characterized uptake of thiamine and demonstrated that this system was also electroneutral, worked as a proton exchanger, and could be inhibited by cations like choline, NMN, tributylmethulammonium (TBMuA) and vecuronium (25). Thus, at least two sodium-independent carriers for cations were present in the basolateral membrane of hepatocytes. With respect to cationic drugs, it was concluded that the relatively small type I organic cations like TEA and TBMuA were transported by a system that was inhibited by choline but not by cardiac glycosides or bile acids. The bulky type II cations like vecuronium on the other hand were transported by a system that was not affected by choline but could be inhibited by cardiac glycosides and bile acids (26) (Figure 4).

Again, the molecular identity of the involved transport systems could not be explained by studies performed in isolate vesicles because these membrane vesicles contained two very similar transport systems with overlapping substrate specificities. To characterize these two systems in detail they needed to be isolated and studied alone, without interference of their functional relatives.

Functional expression cloning

In order to characterize individual transport systems, they had to be isolated and expressed in a recombinant system. Functional expression cloning using *Xenopus laevis* oocytes had been successfully applied to the isolation of the sodium-dependent glucose transporter from rabbit small intestine (27). Functional expression cloning allowed to use a function, e.g. sodium-dependent glucose transport, to go from a complete set of transporters that are expressed in enterocytes to a single transport protein that transports glucose in a sodium dependent way. Because based on the function a cDNA was isolated, the amino acid sequence could be deduced and compared to the amino acid sequences of proteins with similar function. This allowed to determine whether the different proteins were homologous and had a common ancestor. In

brief, mRNA was isolated from the organ of interest, e.g. the rabbit small intestine, and size fractionated to enrich for the function of interest. From this functionally tested size-fraction, cDNA was synthesized and a plasmid library was constructed. This plasmid library was then sub-fractionated, from each sub-fraction cDNA was isolated and transcribed into mRNA. This so-called cRNA was then injected into oocytes and tested for sodium-dependent glucose transport. As soon as a positive fraction was identified it was further subfractionated using the same approach until a single clone was isolated. This single clone then could be further characterized e.g. using a mammalian expression systems, and its amino acid sequence could be compared to all entries in GenBank. Following the same procedure a sodium-dependent bile acid uptake system was cloned from rat liver (28) and identified to be the previously characterized electrogenic uptake system that preferentially transported conjugated trihydroxylated bile acids (Figure 2). A few years later the first organic anion transporting polypeptide Oatp1a1 (*Slc21a1*) was cloned from rat liver as a sodium-independent bile acid and BSP transporter (Figure 2) (29). In the same year the first organic cation transporter OCT1 (*Slc22a1*) was cloned from rat kidney based on N-methylnicotinamide inhibitable TEA uptake (30). Shortly thereafter two independent laboratories reported the cloning of the first organic anion transporter from rat kidney using PAH uptake as their functional assay, and named it ROAT1 and OAT1, respectively (*Slc22a6*) (31,32).

The following paragraphs will briefly summarize the characteristics of the cloned drug uptake transporters expressed in hepatocytes (Figure 5) and in the proximal tubule (Figure 6).

Sodium/taurocholate cotransporting polypeptide (NTCP)

Rat Ntcp, a glycoprotein of 362 amino acids was the first Ntcp cloned from rat liver. Orthologous proteins were subsequently isolated from several different species including man, mouse and rabbit and shown to be exclusively expressed at the basolateral membrane of hepatocytes. Their function was characterized in different recombinant systems. The functional characteristics of the sodium-dependent bile acid transporter characterized in the perfused liver, in isolated hepatocytes and isolated membrane vesicles were all confirmed (33). Recently it could even be demonstrated that human NTCP can transport the cholesterol lowering rosuvastatin (34) and thus potentially can contribute to overall drug uptake into hepatocytes.

Organic anion transporting polypeptides (humans OATPs, rodents Oatps)

Oatp1a1 (previous gene symbol *Slc21a1* current gene symbol *Slco1a1*) was the first of the OATPs/Oatps cloned from rat liver as a sodium-independent BSP and bile acid uptake system (29). Several groups identified additional OATPs/Oatps and today the sequences of more than 160 different members of the OATP superfamily in over 25 animal species have been deposited in or predicted by GenBank. Humans have 11 OATPs, some of which are involved in sodium-independent multispecific uptake of endo- and xenobiotics. They can transport bilirubin, BSP, bile acids, numerous drugs, and even chemotherapeutic agents, and they can be inhibited e.g. by indocyanine green (2,5,35). Several of these OATPs play a major role in drug uptake into the liver and the kidney.

OATP1A2

OATP1A2 (*SLCO1A2*) is a glycoprotein of 670 amino acid that has been shown to be expressed in the brush-border membrane of the distal nephron (36) (Figure 7). It is also expressed in the small intestine, in biliary epithelial cells and in endothelial cells of the blood-brain barrier. OATP1A2 is a polyspecific transporter that has been characterized in a number of experimental systems as a sodium-independent transporter for estrone-3-sulfate and taurocholate. Besides these two model substrates, OATP1A2 can transport a wide range of endogenous as well as exogenous mainly amphipathic organic compounds including bile salts, eicosanoids, hormones and their conjugates, cyclic and linear peptides, toxins, even some organic cations, and

numerous drugs (5). With its expression at the brush-border membrane of the proximal tubule OATP1A2 is probably involved in renal reabsorption of endo- and xenobiotics.

OATP1B1

OATP1B1 (*SLCO1B1*) was the first liver specific OATP cloned and represents a 691 amino acid glycoprotein. It seems to be exclusively expressed at the basolateral membrane of hepatocytes (Figure 5) where it plays an important role in the uptake of a wide variety of compounds include bilirubin, BSP, bile salts, hormones and their conjugates, cyclic and linear peptides, and several drugs like antibiotics, angiotensin receptor antagonists, ACE inhibitors, statins, and anticancer drugs (5). Several polymorphisms have been described (35) and a genome-wide association study linked *SLCO1B1* polymorphisms to an increased risk of statin-induced myopathy (37) supporting the important role of OATP1B1 in the hepatic uptake of statins and possibly of additional drugs.

OATP1B3

OATP1B3 (*SLCO1B3*), a 702 amino acid glycoprotein, is the second liver-specific OATP that is expressed in the basolateral membrane of hepatocytes (Figure 5). In addition, OATP1B3 has also been shown to be expressed in certain gastrointestinal cancers (2). Different experimental systems have been used to characterize this transporter and substrates including BSP, bile salts, hormones and their conjugates, eicosanoids, cyclic and linear peptides, toxins, and numerous drugs including several anticancer drugs have been identified (5). Thus OATP1B3 might play an important role in the uptake of endo- and xenobiotics into hepatocytes as a backup system for OATP1B1, and it could also be involved in the uptake of drugs or hormones into cancer cells. However, its exact role in cancer needs to be further investigated.

OATP2B1

OATP2B1 (*SLCO2B1*) is the third OATP expressed in the basolateral membrane of hepatocytes (Figure 5). It is also expressed in the small intestine, the heart, the blood-brain barrier and the placenta. OATP2B1 is a glycoprotein of 709 amino acids that was originally cloned from a human kidney cDNA library, but it is not clear what its role in the kidney is. Besides the model substrates BSP and estron-3-sulfate, it has been shown to transport dehydroepiandrosterone sulfate. At low pH uptake of several additional compounds including taurocholate, fexofenadine, statins, glibenclamide, and the loop diuretic M17055 has been documented (5). Since the pH in portal blood is not supposed to be acidic, the role of OATP2B1 in the uptake of drugs into hepatocytes is most likely limited. Taken together, based on the functional characterization and the expression pattern of these three OATPs, we are able to begin to determine the role that each individual transporter plays. The development of knockout mice, in which single individual genes are inactivated, might help to further characterize the physiological role these transporters play e.g. in overall bile acid homeostasis or in the pharmacokinetics of drug disposition. However, although an Oatp1b2 knockout mouse has been characterized (38) conclusions to the human situation are not as straightforward because a different set of OATPs is expressed in human liver (OATP1B1, OATP1B3, OATP2B1) as compared to mouse liver (Oatp1a1, Oatp1a4, Oatp1b2, Oatp2b1). Humanized mice, i.e. mice that are missing all the murine Oatps but express a certain set of human OATPs, could help to advance this field tremendously. However, when e.g. pharmacokinetic experiments are compared to the human situation, it has to be kept in mind that all drug metabolizing enzymes and plasma binding proteins still are mouse proteins. Thus, although humanized mice have a great potential, their use in the elucidation of the function of human transporters remains to be established.

Organic cation transporters (OCTs)

The first OCT, OCT1 was isolated by expression cloning from rat kidney (30) based on NMN inhibitable TAE transport. This transport was also inhibited by the classical cyanine 863 and by several additional established cation transport inhibitors. Based on northern blot analysis strong renal expression was demonstrated. However, the human orthologue is mainly expressed in the liver (39) (Figure 5). Subsequently, several additional cation transporters were identified and classified within the *SLC22A* superfamily (1).

OCT1

OCT1 (*SLC22A1*) is a glycoprotein of 553 amino acids that is most strongly expressed at the basolateral membrane of human hepatocytes (Figure 5) where it can mediate uptake of numerous organic cations. It has been shown to transport the model substrates TEA, N-methylquinine, MPP, as well as drugs like desipramine, aciclovir, ganciclovir, metformin, and even endogenous compounds like serotonin and certain prostaglandins (1,39). OCT1 seems to play an important role in the hepatic elimination of endogenous and exogenous cationic compounds.

OCT2

Human OCT2 (*SLC22A2*) is a glycoprotein of 555 amino acids that was cloned as a homologue of rat Oct2. It is expressed in the proximal tubule at the basolateral membrane (Figure 6) and has been shown to transport endogenous cations like choline, acetylcholine, dopamine and histamine as well as drugs like the histamine receptor antagonists cimetidine and ranitidine, the antidiabetic metformin, the anticancer drug cisplatin, the antihypertensive debrisoquine, and the antimalarial quinine (1,39). Human OCT2 thus seems to play an important role in mediating the first step in the renal excretion of numerous organic cations.

OCT3

Although the 556 amino acid human OCT3 (*SLC22A3*) is expressed in the basolateral membrane of the proximal tubule (Figure 6) where it might be involved in the renal elimination of organic cations, its major role seems to be outside the kidneys. OCT3 is expressed at the sinusoidal membrane of hepatocytes (Figure 5) but its functional role in the biliary excretion of organic cations remains to be established. Furthermore, it is expressed in the placenta, in the central nervous system and in the heart where it is involved in neurotransmitter transport (1).

OCTN1

OCTN1 (*SLC22A4*) is a 551 amino acid protein that is expressed at the brush-border membrane in the proximal tubule (Figure 6). It is also strongly expressed in the trachea, in skeletal muscle and in bone marrow. OCTN1 is a polyspecific organic cation transporter that mediates transport of various organic cations including TEA, quinidine, pyrilamine, verapamil and L-carnitine. The transport mechanism is that of electroneutral H⁺/organic cation exchange, and cations can be transported in both directions. OCTN1 is inhibited by numerous other organic cations like choline, cimetidine, lidocaine, procainamide and quinine. Under physiological and/or pharmacological conditions it seems to be involved in the renal secretion of several organic cations but also in the reabsorption of the antioxidant ergothioneine (1,39).

OCTN2

OCTN2 (*SLC22A5*) is a 557 amino acid protein expressed in the liver (Figure 5), the kidney (Figure 6), in skeletal muscle, in the heart and in placenta. It is a Na⁺-dependent high affinity L-carnitine transporter that can also transport acetyl-L-carnitine and the β -lactam antibiotic

cephaloridine in a sodium-dependent way. Besides being a sodium-dependent transporter, OCTN2 is also a polyspecific sodium-independent organic cation transporter that can mediate the uptake of TEA, choline, verapamil and purilamine. OCTN2 is responsible for L-carnitine uptake into the proximal tubule cells and mutations that affect its function or expression can lead to the disease called “systemic carnitine deficiency” which is a disorder of the mitochondrial fatty acid oxidation (1,39). The role of OCTN2 as drug uptake system in the liver however, remains to be established.

Experiments with knockout mice for Oct1, Oct2, Oct3 and double knockouts for Oct1 and Oct2 (40) indicated that Oct1 is important for the excretion of organic cations (TEA) in the liver while Oct1 and Oct2 together are important for renal secretion of TAE. Oct3 seems most important in heart and in embryonic tissues. Furthermore, it seems to be critical for the regulation of salt intake (41).

Organic anion transporters (OATs)

Besides the organic cation transporters, the *SLC22A* superfamily also contains the organic anion transporters (OATs). OAT1 was the first of these transporters that was cloned from rat kidney as OAT1 or ROAT1 (*Slc22a6*) based on probenecid inhibitable PAH uptake (31,32). Additional OATs were later identified in numerous epithelial tissues of several different species and today at least 6 functionally characterized human OATs are known (42,43).

OAT1

OAT1 (*SLC22A6*) is a 550 amino acid glycoprotein which is expressed at the basolateral membrane of the proximal tubule (Figure 7) and in the choroid plexus. It is involved in the renal secretion of a multitude of organic anions including PAH. PAH is taken up via OAT1 in exchange for the endogenous intracellular α -ketoglutarate. Functionally, OAT1 has been characterized in several experimental systems as a multispecific exchanger that can transport endogenous compounds like prostaglandin E_2 and $F_{2\alpha}$, cAMP, cGMP, folate and urate, but also numerous drugs including antibiotics, antivirals, H2 blockers, diuretics, NSAIDs, statins and uricosurics (1,42).

OAT2

OAT2 (*SLC22A7*) is a 546 amino acids protein that is expressed in humans at the basolateral membrane of the proximal tubule (Figure 7) while it is expressed at the brush-border membrane in mice and rats (42). Besides in the kidney, OAT2 is also expressed at the basolateral membrane of hepatocytes (Figure 5). It is involved in hepatocellular uptake and renal secretion of endogenous organic anions including cAMP, dehydroepiandrosterone sulfate, estrone-3-sulfate, prostaglandins, ascorbate and α -ketoglutarate. OAT2 also transports compounds of the same drug classes that are substrates for OAT1, but fewer compounds have been tested. With respect to anticancer drugs, OAT2 is a high-affinity transporter for 5-fluorouracil and taxol (42).

OAT3

OAT3 (*SCL22A8*) is the third (second in rodents) basolateral OAT (Figure 7) and has 542 amino acids. Like OAT1, it is also expressed in the choroid plexus. OAT3 has also been characterized as an exchanger that can exchange intracellular α -ketoglutarate for numerous organic anions. Substrates of OAT3 include the endogenous compounds cAMP, cortisol, dehydroepiandrosterone sulfate, estrone-3-sulfate and even the OATP model substrate estradiol-17 β -glucuronide. Similar to OAT1 and OAT2, various drugs have been shown to be transported by OAT3. In particular, OAT3 is able to transport the cationic cimetidine,

famotidine, and ranitidine while OAT1 only seems to transport the uncharged form of cimetidine (1,44).

OAT4

OAT4 (*SCL22A11*) is a 550 amino acid protein that is specific to humans. It works as an asymmetric anion exchanger that transports glutarate and α -ketoglutarate only out of the cells. OAT4 is expressed at the brush-border membrane of the proximal tubule (Figure 7) where it is either involved in the reabsorption of organic anions or in the secretion of organic anions that have been taken up into the proximal tubule cells via basolateral OATs. OAT4 is also expressed in the placenta. Substrates of OAT4 are endogenous organic compounds like estron-3-sulfate, dehydroepiandrosterone sulfate and prostaglandins. Although it is a multispecific transporter, OAT4 has a more limited substrate specificity than other OATs, but it transports xenobiotics like tetracycline, zidovudine, methotrexate, bumetanide, ketoprofen and salicylate (1,42).

OAT7

The liver specific OAT7 (*SLC22A9*) has only recently been characterized as a multispecific organic anion transporter (43). It is a 554 amino acid protein that is expressed at the basolateral membrane of hepatocytes (Figure 5). When expressed in *Xenopus laevis* oocytes, OAT7 transported the sulfated hormones estron-3-sulfate and dehydroepiandrosterone sulfate but none of the typical substrates of organic anion or cation transporters (43). OAT7 transport was not inhibited by the prototypical OAT inhibitor probenecid and it turned out that OAT7 could transport the short chain fatty acid butyrate. With its narrow substrate specificity and its ability to transport butyrate OAT7 is a novel and unique organic anion transporter whose role in the elimination of xenobiotics remains to be determined.

Knockout mice have been generated for Oat1 and Oat3 (40). Both types of mice are fertile and normal but they have reduced excretion or transport of several organic anions. Because the function of Oat1 and Oat3 is similar, it seems that when Oat1 is missing normal development is possible because Oat3 works as a backup system and vice versa.

Structure-Function relationship experiments

Ideally, X-ray crystallography would be used to determine the three dimensional structures of a transport protein. However, because so far none of the OATPs, OCTs or OATs have been crystallized, comparative homology modeling was used to generate three dimensional structures for OATP1B3 (45), OCT1 (46), OAT1 (47) and OCT2 (48). These models were calculated based on the crystal structures of the lactose permease or the glycerol-3-phosphate transporter from *E. coli*. In combination with data obtained from experiments with chimeric proteins, site-directed mutagenesis and cysteine scanning mutagenesis, valuable information was obtained towards a better understanding of structure function relationships for these drug transporters. For OCT1, several amino acid residues have been identified to be involved in substrate binding. Based on the calculated homology model OCT1 has a substrate binding region rather than a single binding site and consequently more than one compound could bind in the region to OCT1 at the same time (39). Additional recent experiments demonstrated that several amino acids located within a cavity can be accessible from both, the extracellular and the intracellular side of the plasma membrane and thus are likely part of the translocation pathway (49). Overall, the currently available data suggest, that OCT1 is polyspecific because substrates can interact, even at the same time, with a binding region or cavity instead of a discrete site.

Experiments with chimeras of OATP1B3 and OATP1B1 followed by site-directed mutagenesis revealed that several amino acids in transmembrane domain 10 of OATP1B3 are important for binding and/or transport of cholecystokinin-8 (CCK-8). Docking experiments identified additional amino acid residues in transmembrane domains 4 and 7 that could be involved in CCK-8 transport (50). Similar studies for OATP1B1 suggested that transmembrane domains 8 and 9 (51) and 10 (52) are important for OATP1B1-mediated transport. Given that these OATPs are also polyspecific transporters and that a putative model was obtained based on the same *E. coli* transporters as for OCT1, it is very likely that the binding sites of the multispecific OATPs also consists of a region rather than of a discrete single site and can accommodate the numerous structurally unrelated substrates. Functional data that demonstrate substrate dependent inhibition further support this concept but additional experiments are required to elucidate the underlying molecular mechanisms.

Future studies

Structure

Although comparative modeling together with site-directed mutagenesis has revealed some insight into the three dimensional structure of OATPs, OCTs and OATs, detailed information is still missing and the structures of these transporters have to be determined using crystallography. As a first step towards this goal rat Oct1 has been purified and functionally reconstituted from insect cells and in a cell free expression system. Such functional reconstitution of the purified transporters is a prerequisite for the successful elucidation of the tertiary structure of these transporters and several laboratories are currently working towards this goal.

Genetically modified mice

Knockout mice for several Octs, Oats and for Oatp1b2 have been published and additional Oatp-knockout mice are currently characterized (40). However, given that there are species differences, humanized mice, i.e. mice that do not express the intrinsic murine transporter but express the human transporter as a transgene (54) are promising tools to determine the physiological and pharmacological roles of the individual drug transport systems. However, it has to be kept in mind that e.g. plasma proteins and drug metabolizing enzymes still are murine proteins and thus extrapolation of pharmacokinetic behavior of certain drugs to the human situation might not be straight forward because of the differences in these proteins.

Drug-drug and drug-food interactions

With the detailed characterization of the substrate specificities of these drug transporters it became clear that certain drug-drug and drug-food interactions (e.g. statins and grapefruit juice) occur at the transporters (55). Cell based high throughput screening assays that are currently developed in different laboratories should allow to identify novel substrates and inhibitors for the different transporters and will be the basis for structure activity relationship experiments to better define the substrate binding and transport sites and to prevent potential adverse interactions.

Computer models

Pharmacokinetic computer models developed with the help of kinetic constants that are obtained with the cloned transporters will allow predicting how drugs and other xenobiotics will be handled e.g. by the kidneys or the liver and what role each of these two organs might play in the elimination of the respective chemicals. 3D-QSAR studies are needed for the different substrate binding sites and will yield information about the exact substrate characteristics that need to be fulfilled in order for a chemical to be a substrate or an inhibitor

of the drug transporter studied. Furthermore, such QSAR studies should also result in information that can be used to screen chemical libraries *in silico* for new and specific inhibitors and perhaps even to identify new chemical entities that can be targeted to the kidneys or the liver with the help of one of these drug transporters.

Cancer

Several of these drug uptake transporters are expressed in cancers and several are able to transport anticancer drugs. We don't know whether expression of these drug transporters results in a growth advantage of the cancer because cancer cells might use these transporters for uptake of growth factors or hormones. We also don't know whether these transporters might be used to more efficiently target anticancer drugs to cancer cells and kill the cancer. Thus, the roles these transporters play in cancer need to be determined in order to potentially use them to treat cancer.

Although we have come a long way from the first characterization of drug transport in whole animals and intact organs, there still is a long way to go until we will be able to reconstruct all the transporter features of a proximal tubule cell or a hepatocyte based on the information obtained from studies on individual transport proteins.

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Figure 1. Proposed transport systems for organic anions in the proximal tubule

At the basolateral membrane, the Na⁺/K⁺-ATPase generates the driving force for the Na⁺-dependent uptake of α-ketoglutarate (α-KG) which in turn provides the driving force for α-KG/organic anion (OH⁻) exchange. paminohippurate (PAH) is a model substrate that has been used to characterize this transporter which is inhibited by probenecid. At the brush-border membrane organic anion (urate) uptake is driven by the intracellular alkalinization via the sodium/proton exchanger.

Figure 2. Proposed organic anion and bile salt transport systems in the hepatocyte

The basolateral Na^+/K^+ -ATPase provides the sodium gradient for the sodium-dependent uptake of mainly conjugated trihydroxylated bile acids (BA^-). Sodium-independent uptake of mainly unconjugated bile acids can be stimulated by dicarboxylates like α -ketoglutarate ($\alpha\text{-KG}$) or other organic anions (OA^-). There also is a Na^+ -dependent dicarboxylate [$\text{R}-(\text{COO}^-)_n$] uptake system which provides the driving force for dicarboxylate/bile acid (BA^-) exchange. A 49 kDa protein has been suggested to be the sodium-dependent and a 54 kDa protein to be the sodium-independent bile acid transporter.

Figure 3. Proposed organic cation transport systems in the proximal tubule

Transport of organic cations (OC⁺) across the basolateral membrane is facilitated by an electrogenic transporter. Across the brush-border membrane an organic cation/proton exchanger and an ATP-dependent organic cation transporter have been suggested.

Figure 4. Proposed organic cation transport systems in hepatocytes

At the basolateral membrane an N-methylnicotinamide (NMN⁺)/proton exchanger has been demonstrated. Uptake of small type I organic cations (OC⁺) could be inhibited by choline but not by bile acids or cardiac glycosides while uptake of the larger type II organic cations was inhibited by bile acids and cardiac glycosides but not by choline.

Figure 5. Cloned and characterized organic anion and cation uptake systems in hepatocytes
 In human hepatocytes at the basolateral membrane the sodium-dependent bile acid uptake system is represented by the Na⁺/taurocholate cotransporting polypeptide (NTCP) while the sodium-independent bile acid and organic anion uptake system is made up of several transporters with overlapping substrate specificities: the organic anion transporting polypeptides 1B1 (OATP1B1), 1B3 (OATP1B3), and 2B1 (OATP2B1), and the organic anion transporters OAT2 and OAT7. For organic cations OCT1, OCT3 and OCTN2 have been localized to the basolateral membrane. At the canalicular membrane ATP-dependent efflux transporters are present for bile acids (Bile Salt Export Pump, BSEP), for organic cations (Multidrug resistance protein 1, MDR1), and for organic anions (Multidrug resistance associated protein 2, MRP2).

Figure 6. Cloned and characterized organic cation transporters in the proximal tubule

At the basolateral membrane organic cation transporter 2 (OCT2) and 3 (OCT3) are present. At the brush-border membrane OCTN1 and OCTN2 work together with the ATP-dependent efflux transporter multidrug resistance protein 1 (MDR1).

Figure 7. Cloned and characterized organic anion transport systems in the proximal tubule

At the basolateral membrane the Na⁺/K⁺-ATPase provides the sodium gradient for the sodium-dependent uptake of α-ketoglutarate (α-KG) via the sodium dependent dicarboxylate transporter 3 (NADC3). The intracellular α-KG in turn can drive uptake via organic anion transporters 1 (OAT1) and 3 (OAT3). The driving force and transport mechanism for OAT2 is still under investigation. At the brush-border membrane the sodium-monocarboxylate transporter (SMCT) transports lactate into the cell where it can be used in exchange for urate by the urate transporter 1 (URAT1). The sodium-dependent dicarboxylate transporter 1 (NADC1) mediates uptake of dicarboxylates that can drive OAT4-mediated exchange of organic anions (OA⁻). Organic anion transporting polypeptide 1A2 can mediate the uptake of larger mainly amphipathic organic anions into the proximal tubule cell across the brush-border membrane.