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Perspectives - Minireview

Toxicological Relevance of Transporters

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Transporters are membrane proteins that mediate the transfer of substrate across the cellular membrane. In this overview, the characteristics and the toxicological relevance were discussed for various types of transporters. For drug transporters, the overview focused on ATP-binding cassette transporters and solute carrier family 21A/22A member transporters. Except for OCTN transporters and OATP transporters, drug transporters tend to have broad substrate specificity, suggesting drug-drug interaction at the level of transport processes (e.g., interaction between methotrexate and non-steroidal anti-inflammatory agents) is likely. For metal transporters, transporters for zinc, copper and multiple metals were discussed in this overview. These metal transporters have comparatively narrow substrate specificity, except for multiple metal transporters, suggesting that inter-substrate interaction at the level of transport is less likely. In contrast, the expressions of the transporters are often regulated by their substrates, suggesting cellular adaptation mechanism exists for these transporters. The drug-drug interactions in drug transporters and the cellular adaptation mechanisms for metal transporters are likely to lead to alterations in pharmacokinetics and cellular metal homeostasis, which may be linked to the development of toxicity. Therefore, the transporter-mediated alterations may have toxicological relevance.

Key words: Transporter, Toxicity, Metal transporter, ATP-binding cassette transporter, Solute carrier family transporter.

INTRODUCTION

Transporters are membrane proteins that facilitate the movement of substrates from one location to another, typically across the cell membrane of epithelial/endothelial cells (e.g., the kidney epithelium, the endothelium of the brain microvessel). These proteins are routinely subdivided into two major categories by their dependence on energy: active transporters [i.e., requires energy source, e.g., ATP-binding cassette (ABC) transporters] and facilitated transporters (i.e., do not require an energy source, e.g., GLUT-1). Although a substantial amount of work must be completed for the adequate delineation of the biophysical mechanism(s) of their function, it is quite clear that transporters are often involved in the absorption, distribution and/or disposition of the substrates in the body. That is, depending on the function of the transporter, the substrate may be taken up by the cell/tissue (e.g., SLC 21A family transporters) or may be secreted into the bodily fluids (e.g., MDR1) (Fig. 1). As a result, the transporter may primarily govern the concentration in the tissue for certain substrates. Since the tissue concentration of a drug, environmental toxin or metal, is likely to be related to its pharmacological activity or toxicity, those transporters may be considered to have pharmacological/toxicological significance. However, the toxicological implication was primarily studied using animal models and in vitro experimental systems in the literature. In addition, although human data exist for certain transporters (e.g., SLC21A transporters), a direct correlation on the development of toxicity as a result of the transporter has to be demonstrated. In this forum, I will present an over-

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List of nonstandard abbreviations used: Pgp, P-glycoprotein; MDR, multidrug resistance; MRP, multidrug resistance protein; ABC, ATP binding cassette; GLUT, glucose transporter; SLC, solute carrier; OAT, organic anion transporter; NSAIDs, nonsteroidal antiinflammtory drugs; CSF, cerebrospinal fluid; OCT. organic cation transporter; OATP, organic anion transporting polypeptide; OCTN, carnitine transporter; ZIP and ZnT, zinc transporter; CTR, copper transporter; DMT, divalent metal transporter; HEK293 cells, Human embryonic kidney 293 cells; IRE, iron responsive element

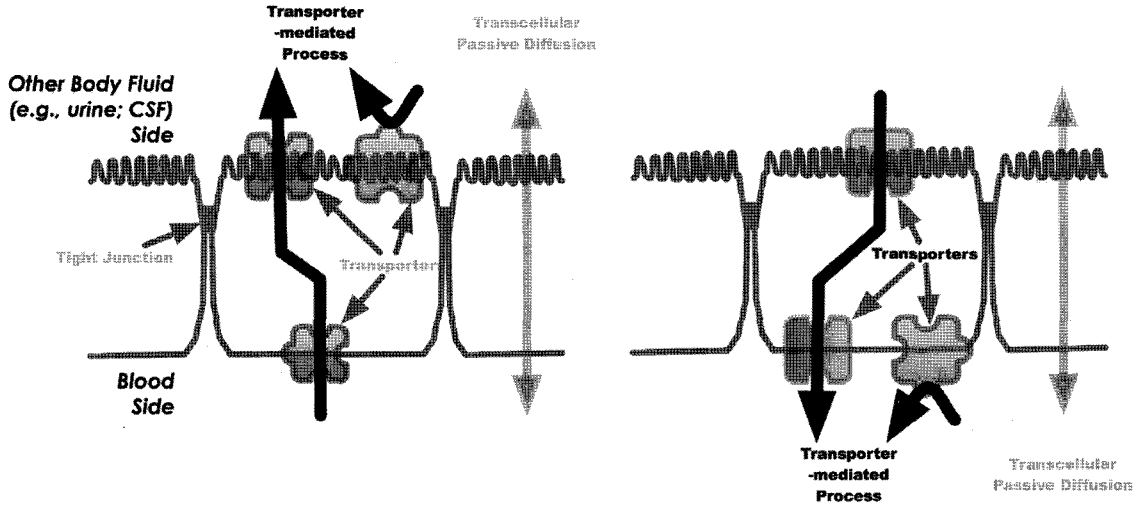

Fig. 1. Schematic representation of transcellular transport mediated by transporters. In general, the transcellular transport to "blood side" indicates absorptive movement while the transport to "body fluid side" represents secretory movement.

view for the characteristics of representative transporters and their toxicological relevance. For the purpose of the presentation, transporters are divided into two parts [one for the carriers of drugs/environmental toxic agents (Drug Transporter) and the other for the carriers of metals (Metal Transporter)] and their significance discussed.

DRUG TRANSPORTERS

Pharmacokinetics is determined by a sum of kinetics processes involved in the absorption, distribution and excretion of a drug. Kinetics, in turn, is primarily governed by the transport processes at the barriers between systemic circulation and tissue (e.g., the blood brain barrier). Previously, the physicochemical properties were traditionally believed to be the major determining factor for pharmacokinetics. However, with recent advances in transporter researches, the original view has been significantly modified. A number of multi-specific transporter families have been identified and characterized (see below). More importantly, these transporters were found to be the primary determinant for the pharmacokinetics in experimental animals as well as in humans.

ATP-Binding cassette transporters.

P-glycoprotein (ABCB1): MDR1, an alias for P-glycoprotein, was originally found in the plasma membrane of multidrug resistant tumor cells. This efflux transporter has two intracellular ATP-binding sites and is a major determinant in drug absorption and in the penetration of substrates across the blood brain barrier (i.e., brain microvessel endothelial cells). The protein is localized in the apical (i.e., brush border) membrane of a number of epithelia (e.g., the intestine, kidney and liver) and the endothelium of the brain microvessel (Table 1). MDR1 is known to have broad substrate specificity for hydrophobic neutral or cationic compounds. Representative substrates for this transporter include digoxin, indinavir, nelfinavir, verapamil and fexophenadine. Recent reviews (Kusuhara and Sugiyama, 2004; Beringer and Slaughter, 2005) on the primary active transporter are also available.

Because the transporter functions as an efflux pump in the interface between the systemic circulation and the organ, the entry of the substrates is likely to be significantly limited in the barrier that expresses MDR1. Co-administration of quinidine (MDR1 inhibitor) and loperamide (MDR1 substrate) resulted in a significant respiratory depression in health volunteers, without any alteration in the plasma concentrations (Sadeque *et al.*, 2000). When administered separately, there was no apparent side effect for these drugs. Therefore, it has been postulated that the co-administration may lead to an interaction of the drugs via MDR1-mediated efflux process, thereby leading to a toxicological outcome (Beringer and Slaughter, 2005). Since the transporter is known for broad substrate specificity, similar toxicological consequences are likely. Disease states may also affect the function of the efflux transporter. For example, Song *et al.* (2003) reported that an experimental hepatic injury led to a significant increase in the protein level and the function of MDR1 in rats. In addition, an experimental hyperglycemic condition was associated with the increase in the expression and the function of the efflux transporter in the rat brain (unpublished

Table 1. Summary of characteristics of ATP-binding cassette and solute carrier family transporters

Name	Gene symbol	Tissue distribution	Membrane localization	Transport mechanism	Representative substrates
ABC transporter P-glycoprotein					
MDR1	*ABCB1*	b, li, k, si	CM(li), BBM(si), LM(bc)	primary active	digoxin, indinavir, nelfinavir, verapamil, fexophenadine
Multidrug resistance-associated protein (MRP) family					
MRP1	*ABCC1*	ubiquitous	ND	primary active	methotrexate, glucuronide, sulfate and glutathione conjugates
MRP2	*ABCC2*	k, li, si, b	ND	primary active	furosemide, indomethacin, methotrexate, vinblastine
MRP3	*ABCC3*	k, li, si, c, lu, p	ND	primary active	anionic glucuronide/glutathione conjugates, methotrexate
MRP4	*ABCC4*	ubiquitous	ND	primary active	adefovir, zidovudine monophosphate, naltrexone
MRP5	*ABCC5*	ubiquitous	ND	primary active	adefovir, mercaptopurine
MRP6	*ABCC6*	ND	ND	primary active	Not determined
Organic anion transporter (OAT) family					
OAT1	*SLC22A6*	k	BLM	ND	adefovir, cidofovir, zidovudine, acyclovir, ganciclovir
OAT2	*SLC22A7*	li, k	BLM	ND	cephalosporins, NSAIDs
OAT3	*SLC22A8*	k	BLM	OA/dicarboxylate antiport	benzylpenicillin, cimetidine, estrone sulfate
Organic cation transporter (OCT) family					
OCT1	*SLC22A1*	li, k,	BLM	facilitative	prostaglandin E_2, prostaglandin F_{2a}
OCT2	*SLC22A2*	k (distal tubule)	BLM	facilitative	amantadine, dopamine, histamine, norepinephrine, serotonin, choline
OCT3	*SLC22A3*	ubiquitous	ND	facilitative	epinephrine, serotonin, tyramine
OCTN family					
OCTN1	*SLC22A4*	ubiquitous	ND	H^+ antiport	quinine, verapamil, carnitine
OCTN2	*SLC22A5*	ubiquitous	ND	Na^+ symport	carnitine, choline
OATP family					
OATP-A	*SLC21A1*	b, k, li, lu, t	LM/AM(bc)	ND	bile salts, fexofenadine
OATP-B	*SLC21A9*	ubiquitous	SM(li), BBM(si)	ND	estrone sulfate, benzylpenicillin
OATP-C	*SLC21A6*	li	BLM	ND	bile salts, pravastatin, atrovastatin, rosuvastatin
OATP-D	*SLC21A11*	ubiquitous	ND	ND	prostaglandin, benzylpenicillin
OATP-E	*SLC21A12*	ubiquitous	ND	ND	taurocholate, triiodothyronine, prostaglandin
OATP-F	*SLC21A14*	b, t	ND	ND	tyroxine, bromosulfophthaleine
OATP-8	*SLC21A8*	li	BLM	ND	eicosnoids, iloprost

Key: (Tissue distribution) k, kidney; li, liver; lu, lung; b, brain; si, small intestine; t, testis; p, pancreas; bc, brain capillary; (Membrane localization) AM, abluminal membrane; LM, luminal membrane; SM, sinusoidal membrane; CM, canalicular membrane; BLM, basolateral membrane; BBM, brush border membrane; (General) ND, not determined.

observation in this laboratory). Therefore, the patients having these diseases may be associated with an exaggerated response via the transport related drug interaction and potentially have an increased risk for an adverse reaction.

Multidrug resistance associated protein (ABCC subfamily): Recently (Kusuhara and Sugiyama, 2004), eight different members of the ABCC subfamily had been cloned (i.e., MRP1-8). MRP1 was first found as an over-expressed protein in tumor cells having multidrug resistance (Kusuhara and Sugiyama, 2004; Leslie *et al.*, 2001). MRP transporters have two ATP-binding domain, located in the cytoplasm, and mediate the efflux of substrates from the cell. MRP1, and 3, were apparently localized in the basolateral membrane of epithelial cells (Leslie *et al.*, 2001). In contrast, MRP2 was found in the brush border membranes (Leslie *et al.*, 2001). For the case of recently cloned MRPs (i.e., MRP 4-8), their subcellular localization has not been reported in the literature. Table 1 summarizes the tissue distribution, subcellular localization and transport mechanisms for ABCC transporters. Similar to MDR1, MRP transporters have a broad specificity for the substrate. Typically, MRPs transport phase II metabolites (i.e., conjugates with glucuronide, sulfate or glutathione), although some unconjugated form may be subjected to the transporter for certain drugs. Representative substrates (Beringer and Slaughter, 2005) for MRPs include meth-

otrexate, glucuronide, sulfate and glutathione conjugates for MRP1, furosemide, indomethacin, methotrexate and vinblastine for MRP2, anionic glucuronide/glutathione conjugates, methotrexate for MRP3, adefovir, zidovudine monophosphate and naltrexone for MRP4, adefovir and mercaptopurine for MRP5.

Because of the broad specificity for the transporter and the participation of the transporter during the detoxification process (viz, excretion of phase II conjugates via MRPs), in principle, the drug interaction between substrates for MRPs may be plausible. Furthermore, metabolic conjugates of toxic agents are known to be substrates for the transporters. For example, it was indicated that a glutathione conjugate of aflatoxin B_1, a mycotoxin, was transported by MRP1 (Loe *et al.*, 1997). In addition, the glutathione conjugate of metolachlor, an herbicide used in large quantities, is a competitive inhibitor (K_i of 22 μM) for the transport of a high affinity MRP1 substrate (Leslie *et al.*, 2001). Therefore, it appears conceivable that these glutathione conjugates may interact with other MRP1 substrates during the transport process. Similarly, flavonoid and nitrosamine derivatives may also interact with the efflux transporter (Leslie *et al.*, 2001), potentially having similar consequence. Since the transporter mediates the efflux process, the interaction may lead to the accumulation of the conjugates of the toxins in the tissue/organ, thereby interfering with the detoxification pathway. However, experimental confirmation on the potential toxicological outcome is necessary.

SLC22A family transporters.

Organic anion transporters (OAT): Recently, four OAT genes (OAT1-4) had been cloned in humans (Kusuhara and Sugiyama, 2004). In the literature, a wide variety of anionic drugs were reported to use the transporter. Representative substrates (Sweet, 2005) for OATs include non-steroidal anti-inflammatory agents (NSAIDs e.g., acetaminophen, indomethacin, ketoprofen, naproxen and salicylate), b-lactam antibiotics, antivirals (e.g., acyclovir, adefovir, asidothymidine, cidofovir and gancyclovir), methotrexate, neurotransmitter metabolites, uremic toxins, and heavy metals. OAT transporters play an essential role in the renal excretion and detoxification of anionic drugs. Interestingly, the substrate specificity for OATs somewhat coincides with that for MRPs or OATPs (see below), suggesting the transporters may be cooperatively involved in the transcellular transport of these substrates. The transport mechanisms for OATs are not clearly defined, with the exception of that for OAT3 [i.e., probably by Cl^- or HCO_3^- antiport mechanism (Kusuhara and Sugiyama, 2004)]. Tissue distribution and subcellular localization for OATs are listed in Table 1.

It has been reported that an acute renal toxicity may occur when patients received methotrexate along with NSAIDs (Aherne *et al.*, 1978; Miyazaki *et al.*, 2004). Since these drugs are known substrates for OAT transporters, the interaction between NSAIDs and methotrexate during transport via OATs may be related to the renal toxicity. Furthermore, considering the fact that OATs transport structurally diverse drugs, similar toxicity for methotrexate may also be developed when the chemotherapeutics are co-administered with drugs that are subjected to the transporter. The concentration homovanillic acid, an uremic toxin, was reported to be elevated in the CSF of the patients with uremic encephalopathy, ultimately leading to neurological dysfunction (Moe and Sprague, 1994). The toxicity may be related to the interaction between the toxin and neurotransmitter metabolites, because the toxin and the metabolites may share a common transport pathway via OAT transporters (Sweet, 2005). Similarly, toxic agents, such as ganciclovir, chlorinated phenoxyacetates and mercury, which are subjected to transport via OATs, often are associated with nephrotoxicity and/or neurotoxicity. Considering the tissue distribution profile and subcellular localization, it was speculated that these toxicities might be linked to the action of the OAT transporters (Sweet, 2005; Miyazaki *et al.*, 2004). However, this aspect of OAT transport requires experimental verification.

Organic cation transporters (OCT): The primary function of OCT transporters is to reabsorb and excrete structurally diverse organic cations. In humans, three members of the OCT family have been found and characterized (Kusuhara and Sugiyama, 2004; Beringer and Slaughter, 2005; Table 1). Representative substrates for the transporters (Beringer and Slaughter, 2005; Sung *et al.*, 2005) are procainamide, quinidine, cardiac glycosides, choline, and tacrine. An interesting feature of OCT transporters is the fact that the protein may bi-directionally transport small hydrophilic organic cations. This aspect of directional movement of the organic cation via the transporters may warrant further investigation.

Recently, 22 and 28 genetic variations were reported for OCT1 and OCT2, respectively. Although the majority of the OCT variants are not apparently functionally important, two of the OCT2 variants are reported to have reduced function. Therefore, it is possible that the individual variability may be related to the genetic variation. However, it remains to be determined whether the alteration in the pharmacokinetics is linked to the toxicological consequence for the cationic drugs.

Carnitine transporters (OCTN): Carnitine, a compound that is required in β-oxidation of fatty acids, is transported by solute carrier family transporters (OCTN) (Kusuhara and Sugiyama, 2004; Miyazaki *et al.*, 2004). At least two OCTN transporters were identified in the literature (Table 1). The transport processes are mediated either by a proton exchange mechanism (for OCTN1) or possibly by Na^+-gradient (for OCTN2). Compared with other SLC22A transporters, OCTN transporters accept a relatively small number of compounds (e.g., pyrilamine, quinine, verapamil and carnitine) (Kusuhara and Sugiyama, 2004). Although a number of disease states (e.g., carnitine deficiency, rheumatoid arthritis) may be linked to the genetic variants of OCTN (Miyazaki *et al.*, 2004), toxicological significance for the transport system has not been extensively studied in the literature.

SLC21A Transporters.

The organic anion-transporting polypeptide (OATP) transporter family is another family of organic anion transporters (Kusuhara and Sugiyama, 2004; Beringer and Slaughter, 2005). These sodium-independent transporters mediate the movement of metabolites and drugs across the basolateral membrane of epithelial cells (Table 1). Representative substrates for these transporters (Beringer and Slaughter, 2005) include bile salts, fexofenadine, estrone-3-sulfate, estradiol-17β-glucuronide, pravastatin, atrovastatin, rosuvastatin, prostaglandin, benzylpenicillin and digoxin. Except for OATP1B1 (SLC21A6, previously OATP-C), the characteristics of the remaining OATPs are not well known. A tissue distribution profile and subcellular localization are summarized in Table 1. For the case of OATP1B1, the transporter may have 12 transmembrane domains with 7 N-glycosylation sites and 2 phosphorylation sites (Beringer and Slaughter, 2005).

The primary function of OATP1B1 is to transport substrates into the hepatocyte from the portal circulation. It has been reported that the hepatic distribution of HMG-CoA reductase inhibitors are mediated by this transporter. Therefore, drug-drug interaction at the level of OATP1B1 transport may lead to a decreased hepatic concentration of statins, thereby leading to adverse effects of the drug (Kim RB, 2004). Consistent with this, human volunteers having a reduced function gene for OATP1B1 were reported to have reduced non-renal clearance of a substrate, suggesting an elevated exposure to the drug and, thus, an elevated risk for toxicity development (Nishizato *et al.*, 2003). Similarly, oral bioavailability of fexofenadine, a substrate for OATP1A2, was reduced by grapefruit juice, an inhibitor for the transporter (Dressor *et al.*, 2005). However, it remains to be determined whether the changes in pharmacokinetics is directly linked to the toxicity of these drugs.

METAL TRANSPORTERS

Transporters for zinc.

Zinc plays a variety of physiological roles in the body (Vallee and Auld, 1990) as evidenced by the fact that the metal is a crucial structural component of a protein or a cofactor (for a review see Vallee and Falchuk, 1993). Therefore, the zinc homeostasis may be crucial for the maintenance of normal physiological function in mammals, including humans (Vallee and Auld, 1990; Vallee and Falchuk, 1993). Since zinc is an essential nutrient, the intracellular concentration must be governed by efficient transport system(s). Consistent with the statement, literature information indicates that at least two transporter families, ZIP and ZnT, are related to control of the intracellular zinc concentration (Kambe *et al.*, 2004). These zinc transporters are extremely redundant as shown below, and the physiological relevance needs to be ascertained.

ZIP transporters: Zrt, Irt-like Protein, or ZIP, family transporters mediate the transfer of zinc into the cytoplasm either by influx from extracellular space or transport from intracellular zinc storage compartments to the cytoplasm. The transport may be dependent on an HCO_3^- gradient (Gaither and Eide, 2000), although the driving force needs further verification. ZIP transporters, originally described as *Saccaromyces cerevisiae* and *Arabidopsis thaliana* (Kambe *et al.*, 2004), were also identified in mammals, including humans. Recently, 14 members of the ZIP family transporters, which are categorized into 4 subfamilies, were identified in humans (Kambe *et al.*, 2004) (Table 2). The substrate specificity for ZIPs is apparently somewhat broad, since the transporters may accept other metals such as iron, manganese and cadmium, and, thus, may have a multiple role (Kambe *et al.*, 2004). One of the interesting features for zinc transport is the fact that the expression of the transporters (for both ZIP and ZnT families) is regulated by extracellular zinc concentration (Gitan and Eide, 2000). The speculation is that the zinc dependent regulation of the transporters may be related to the maintenance of zinc homeostasis. This aspect of zinc transport warrants further investigation.

It has been suggested that a number of pathological conditions (e.g., perioral dermatitis, acral erosion, intermittent diarrhea and growth failure in early infancy) (Van Wouwe, 1989) may be related to zinc deficiency, probably mediated by a mutation in hZIP4 transporter (Wang

Table 2. Summary of tissue distribution and subcellular localization of zinc transporters

Name	Subfamily	Tissue distribution	Subcellular localization
ZIP transporter family			
BAB70848	LIV-1	ND	ND
hZIP4	LIV-1	duodenum, jejunum, colon, stomach, kidney	apical membrane in enterocyte
BIGM103	LIV-1	widely expressed (pancreas)	lysosomal/endosomal compartment
KIAA0062	LIV-1	ubiquitous (liver, pancreas, heart)	ND
KIAA1265	LIV-1	ubiquitous (spinal code, brain)	ND
hLIV-1	LIV-1	breast cancer, prostate, brain, testis	ND
AAH08853	LIV-1	ND	ND
hKE4	LIV-1	ubiquitous (embryo)	endoplasmic reticulum
XP_208649	LIV-1	primary expressed (colon epithelium, adenocarcinoma cells)	ND
hZIP1	II	ubiquitous	plasma membrane in erythroleukemia cells, intracellular vesicles in adherent cells
hZIP2	II	uterus, prostate, monocytes	plasma membrane
hZIP3	II	ND	ND
BAA92100	I	ND	ND
BAC04504	gufA	ND	ND
CDF transporter (ZnT) family			
hZnT-5	II	ubiquitous (pancreas, prostate, ovary, testis)	Secretory granules in pancreas B cells, golgi apparutus
hZnT-7	II	ubiquitous (liver, small intestine, spleen)	golgi apparutus
hZnT-1	II	ubiquitous (small intestine, kidney, placenta)	Plasma membrane, basolateral side
hZnT-6	III	brain, liver, small intestine	trans-Golgi network, vesicular compartments
hZnT-3	III	Brain, testis	Synaptic vesicles
hZnT-2	III	Small intestine, kidney, seminal vesicle, testis, placenta, mammary gland, prostate	Late endosomes
hZnT-8	III	Specifically expressed in pancreas B cells	ND
hZnT-4	III	ubiquitous (mammary gland, brain, small intestine)	Endosomal compartment, trans-Golgi network

Key: ND, not determined.

et al., 2002). Therefore, the functional inhibition of ZIP4, by other metal(s), or other agents, that may interact with the transporter in the intestinal epthielium may lead to a pathological/toxicological outcome similar to that in acrodermatitis enteropathica.

The LIV-1 transporter, one of the human ZIP transporters, is reported to be positively regulated by steroidal hormones (e.g., estrogen and growth factors) in breast cancer cells (El-Tanani and Green, 1997). Therefore, agents that affect the level of steroidal hormones may increase the expression of the zinc transporter in the cell plasma membrane, thereby increasing the intracellular zinc concentration. The alteration in zinc homeostasis may lead to an increased incidence of zinc toxicity.

CDF (ZnT) transporters: ZnT transporters or CDF transporters mediate the transfer of zinc from the cytoplasm, either by the efflux to the extracellular space, or by the transport into the intracellular zinc storage compartments. Recently, eight ZnT transporters were identified in mammals and, subsequently, categorized into two subgroups (i.e., II and III, Table 2). The primary driving force of the transporter is not fully delineated, although there has been an indication that a K^+ and/or an H^+ gradient may be involved (Kambe *et al.*, 2000). Tissue distribution and subcellular localization of ZnT are also summarized in Table 2. Except for ZnT-1 (i.e., localized in the cell plasma membrane), ZnTs are primarily localized in the cytoplasm (Table 2), suggesting that these transporters are responsible for the sequestration of zinc into intracellular zinc storage compartments. Interestingly, ZnT-6 appears to be redistributed from the intracellular domain to the plasma membrane during high zinc exposure (Huang *et al.*, 2002). In addition, mRNA levels for ZnT-1 and 2 were increased by the treatment of the cell with a high zinc concentration (McMahon and Cousins, 1998). The zinc-sensitive redistribution/regulation of ZnTs may be actively involved in the control of the intracellular zinc concentration in the cell.

Vesicular zinc concentration had virtually disappeared in the hippocampus and the cortex of the gene knockout animal for ZnT-3, a ZnT form exclusively expressed in the brain, and the mutant mice were more prone to seizures induced by kainic acid (Cole *et al.*, 2000). Taken together, these observations suggest that the

Table 3. Summary of transport mechanism, tissue distribution and subcellular localization of copper transporters

Name	Aliases	Transport mechanism	Tissue distribution and cellular/subcellular expression
Copper transporter family			
hCtr1	hCtr1, Ctr1	Energy independent and potassium dependent	Ubiquitous mRNA expression; highest in liver, lowest in brain and skeleton muscle. Protein localized to plasma membrane and intracellular vesicles of cultured cells
hCtr2	hCtr2, Ctr2	ND	Ubiquitous mRNA expression

Key: ND, not determined.

zinc homeostasis may have pathological/toxicological consequences. In addition, gene knock-out animals for ZnT-1 or ZnT-4 were either lethal at the embryonic stage (ZnT-1 knock-out) or had other problems (ZnT-4 knock-out, e.g., male specific sudden death) (Kambe *et al.*, 2004). Therefore, agents that interfere with the function of the ZnT transporters may be potentially toxic. However, experimental verification of the hypothesis has not been described in the literature.

Transporter for copper.

Copper, another transient metal, is essential for normal physiology (Dameron and Harrison, 1998) because of the catalytic action of the metal in proteins and other endogenous macromolecules. The concentration of copper at the level exceeding the physiological range often leads to toxicity in the cell, and, ultimately, to the organ. While redundant mechanisms (e.g., metabolic pathways) exist for protection against copper toxicity, only the copper transport system will be presented in this overview. A high affinity transport system for copper (CTR) was first described in *Saccharomyces cerevisiae* (Petris, 2004) and the homologues were later identified in mammals, including in humans (Zhou and Gitschier, 1997). All CTR family transporters, including human forms, have three transmembrane domains (Petris, 2004) forming a homotrimeric complex (Eisses and Kaplan, 2002). Recently, two CTR transporters, hCTR1 and hCTR2, were described in the literature (Petris, 2004). Functional study with an *in vitro* hCTR1 expression system indicates that the high affinity (i.e., K_m of 1~5 μM) transport system primarily drives the copper influx into the cell (Lee *et al.*, 2002). The process does not apparently require an energy source, although a high concentration of potassium (by co-transport or potential gradient mechanism) stimulates the influx (Lee *et al.*, 2002). The other human copper transport system hCTR2, a homologue of yeast CTR2, may be related to the transport of copper from the intracellular copper storage to the cytoplasm (Petris, 2004). However, the biochemical characterization for hCTR2 is far from complete and warrants further investigation. A brief summary of the characteristics of the human CRT transporters is listed in Table 3. Recently, it was reported that an exposure of a high concentration of copper led to the endocytosis and the degradation of hCTR1 in HEK293 cells expressing the transporter. This observation suggests that the copper transporter, at least the hCTR1, may be regulated, which is consistent with the cellular adaptation process. However, the similar in vivo regulation has to be demonstrated to realize the toxicological relevance of the copper transporter.

In addition to CTR family transporters, mammalian copper exporting ATPase has been described (Dameron and Harrison, 1998). However, the biochemical characterization is not complete for the protein and, thus, the ATPase is not included in this overview.

Transporters for multiple metals.

Divalent metal transporter 1 or DMT1 is a remarkably diverse metal transporter in many ways. First, this transporter has a number of aliases in the literature. Along with DMT1 (for a recent review see Garrick *et al.*, 2003), it has been described as Nramps (natural resistance associated macrophage protein 2), DCT1 (divalent cation transporter 1) or SLC11A2 (solute carrier family 11, member 2). For the purpose of this overview, DMT1 will be used.

Second, another diversity of DMT1 is related to the specificity of its substrates. In the literature, it was indicated that up to eight metals (i.e., Fe^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+}) might be transported by the transporter (Gunshin *et al.*, 1997). However, the electrical conductance (i.e., an indirect measurement of the transport), rather than the mass transfer, was measured in the study by Gunshin and co-workers (1997). In more recent studies (Garrick *et al.*, 2003), metal uptake was measured for five representative substrates (i.e., Fe^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}) in DMT1 expressing HEK293 cells. While these metals were indeed transported in the assay system, Zn^{2+} transport could not be measured when the transporter was functionally expressed in HEK293 cells. The literature is not entirely clear on the underlying reason(s) for the discrepancy. A number of experimental variables (e.g., conductance measurement vs. transport measurement; expression in

amphibian cells vs. expression in mammalian cells) may have contributed to the difference, and, thus, additional verification is necessary to fully resolve this issue. Recently, substrate competition study suggests that the zinc ion does not inhibit the flux of $^{54}Mn^{2+}$ in HEK293 cells expressing DMT1 (Conrad *et al.*, 2000), which further supports the possibility that zinc transport is not mediated by the transporter.

In the literature, it was indicated that DMT1 might have multiple isomers. Iron responsive element (IRE) at the 3'-UTR of DMT1 may interact with iron regulatory protein thereby affecting the stability of the resulting mRNA. This indicates that DMT1 exists either with or without an IRE portion [viz, +IRE DMT1 or –IRE DMT1 (Garrick *et al.*, 2003)]. Currently, the literature remains undecided as to which isoform is the predominantly expressed DMT1 in the body (Garrick *et al.*, 2003; Gunshin *et al.*, 1997). In addition to the two isoforms, Hubert and Hentze (2001) reported an alternative splicing in the 5' end of the DMT1 mRNA. Therefore, a total of 4 isomers, ranging in the number of amino acids from 561 to 599, may exist. It is interesting to note that the N-terminal of the new 5'-end variant [i.e., the one identified by Hubert and Hentze (2001)] may contain a candidate sequence for a nuclear localization signal, suggesting that these variants are localized in the cell nucleus. Consistent with this, Garrick *et al.* (2003) reported that the nuclear expression of DMT1 was detected in superior cervical ganglia of neonatal rats. Further verification, as well as the clarification on the functional implication, appears necessary to fully appreciate the importance of the transporter.

The primary function of DMT1 is reportedly to transport iron (Canonne-Hergaux *et al.*, 1999) in the intestinal epithelium. That is, Fe(III) in the gastric fluid may be reduced to Fe(II), by a mechanism yet to be elucidated, and transported into the enterocyte by the transporter (Canonne-Hergaux *et al.*, 1999). Expression of the proton-driven transporter is apparently regulated by iron concentration as evidenced by the fact that the exposure to iron increased DMT1 mRNA (Wang *et al.*, 2002). Interestingly, the -IRE form, but not the +IRE form, was responsive to iron exposure in human bronchial epithelial cells (Wang *et al.*, 2002). The isoform specific regulation by iron was associated with the increased function, suggesting that the DMT1 regulation is related to metal detoxification. In addition to the potential role of DMT1 in metal detoxification, the transporter has been implicated in Mn toxicity (Garrick *et al.*, 2003). Mn, a cytotoxic agent and a substrate for DMT1, may be involved in the neuronal differentiation (Roth *et al.*, 2002). When rat pheochromocytoma cells were pretreated with an iron chelator, the Mn-induced cell death and differentiation was increased, suggesting that the chelation of iron may lead to an increased transport of Mn to the cell. Consistent with this, $^{54}Mn^{2+}$ transport was readily inhibited with the IC_{50} value of ~20 μM (i.e., the iron concentration that is expected to be too high after the iron chelation) by the presence of iron in rat pheochromocytoma cells. Furthermore, the iron chelation was associated with the increased expression for both +/-IRE DMT (Roth *et al.*, 2002). Therefore, managanese toxicity may be directly linked to iron status, probably via the function of DMT1.

CONCLUDING REMARKS

In this overview, various types of transporters were discussed. Because of the diversity of transport systems, a consistent conclusion would not be possible for the toxicological relevance of transporters. For the case of drug transporters, the drug-drug interaction may have toxicological consequence (e.g., interaction between methotrexate and NSAIDs). For the case of metal transporters, similar interaction mechanism may have toxicological significance (e.g., Mn^{2+} transport via DMT1 transporter). In addition, the regulation on the expression of metal transporter may be related to the toxicological consequence. Pharmacogenetics of transporters were not included in this overview, except for OATP and OCT transporters, because the toxicological relevance was not clearly indicated for transporter variants. Currently, this aspect of transporter research is actively studied (Beringer and Slaughter, 2005).

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