Organic and inorganic transporters of the testis: A review

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Abbreviations: ABC, ATP-binding cassette; ADME, absorption distribution metabolism and elimination; AQP, aquaporin; BTB, blood-testis barrier; CT, carnitine transporter; DMT, divalent metal transporter; ENT, equilibrative nucleoside transporter; ES, ectoplasmic specialization; GLUT, glucose transporter; LDH-C₄, lactate dehydrogenase C₄; MATE, multidrug and toxin extrusion; MGT, male genital tract; MRP, multidrug resistance-associated protein; NASH, non-alcoholic steatohepatitis; NHE, sodium-hydrogen exchanger; OAT, organic anion transporter; OATP, organic anion transporting polypeptides; OCT, organic cation transporter; P-gp, P-glycoprotein; SLC, solute carrier; SOAT, sodium-dependent organic anion transporter; TfR, transferrin receptor; ZIP, Zrt-, Irt-, related protein.

Transporters have a huge impact on the toxicology and pharmacological effects of xenobiotics in addition to being implicated in several diseases. While these important proteins have been well studied in organs such as the kidney or liver, characterization of transporters in the testis is still in the early stages. Knowledge of transporter function may greatly advance the field's understanding of the physiological and toxicological processes that occur in the testis. Several foundational studies involving both organic and inorganic transporters have been critical in furthering our understanding of how the testis interacts with endogenous and xenobiotic compounds. This review provides an overview of how transporters function, their clinical significance, and highlights what is known for many of the important transporters in the testis.

Introduction

In order for a drug or a toxicant to have an effect, it must reach the target site at sufficient concentrations. Whether a xenobiotic will reach the target site depends primarily on how well the compound is absorbed, distributed, metabolized and eliminated from the body. These processes, typically referred to as ADME, largely determine the plasma concentrations of a compound and as such, are critical for understanding the toxicity of a compound.^{1,2} One group of specialized proteins called transporters are known to play vital roles in the ADME processes for many clinically relevant xenobiotics.^{1,3,4} Transporters are proteins that span across lipid bilayers and allow for the passage of chemicals through biological membranes. The physiological function of transporters is to allow endogenous substrates access

to cells and biological compartments or to restrict access of potential toxicants within the body.^{5,6} For example, transporters that are expressed in the intestine can either allow for the absorption of ingested compounds into the body, or keep those compounds from entering the bloodstream.^{7,8} Additionally, a compound's interaction with transporters in the liver or kidney can drastically affect the metabolism and excretion of the compound.^{6,9-13}

Nearly all transporters are associated with one of 2 superfamilies based on the driving force: ATP-binding cassette (ABC) transporters that use ATP hydrolysis to provide energy for transport against an electrochemical gradient, and solute carrier (SLC) transporters that transport compounds using electrochemical gradients, typically coupled to a cosubstrate, typically an ion such as Na⁺.^{1,2,14-16} This transport coupling can translocate in the same (symport) or in opposing directions (exchange or antiport). As an example, sodium/hydrogen exchanger family (gene SLC9A) couples the efflux of H⁺ ions with uptake of Na⁺ ions.¹⁷⁻²⁰ Since extracellular Na⁺ concentrations far exceed intracellular concentrations, the uptake of Na⁺ provides a driving force that allows for the efflux of H⁺ even into an acidic environment. Within these 2 superfamilies, transporters are typically named based on their substrates. For example, equilibrative nucleoside transporters (ENT) facilitate the movement of nucleosides, organic cationic transporters (OCT) interact with organic cations etc.^{4,21-24} Some families of transporters, such as ENTs, have a narrow range of substrates while others, such as multidrug resistance-associated proteins (MRP), have a variety of potential substrates.^{2,25-27} Transporters can also be characterized based on the directionality, i.e. whether they allow substrates into cells (uptake), out of cells (efflux), or both (bidirectional).

When investigating transport activities in the context of biological systems, it's critical to know the localization of the protein as it can greatly impact transporter function for a tissue. Localization refers to which types of cells express functional protein and, in polarized epithelial cells, whether the transporter resides in the basolateral membrane or the apical membrane. If an uptake

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transporter for a toxicant is located on a basolateral membrane of an epithelial cell and an efflux transporter is on the apical side, then this would represent a transepithelial pathway, shuttling substrate from the blood into the cell and then out through the apical membrane. Such a transepithelial pathway can explain how a particular toxicant can accumulate in an area of the body. A solid understanding of transporter function and localization can lead to a better prediction of toxicant exposure.

There is a significant body of data that demonstrates the clinical significance of transporters. A classic example is Dubin-Johnson syndrome which is an increase in conjugated bilirubin due to a defect in multidrug resistance-associated protein 2 (MRP2, gene name ABCC2), a canalicular transporter that is responsible for the efflux of bilirubin glucuronides into bile.²⁸⁻³¹ There is also increasing evidence that diseases of the liver, such as nonalcoholic steatohepatitis (NASH), can influence transporter profiles in the liver, which is responsible for eliminating many drugs.^{6,10,32} This alteration can cause altered plasma levels for many commonly prescribed drugs which may lead to toxic side effects even at standard therapeutic doses. Transporters can also be important in mediating drug-drug interactions, for example cimetidine and procainamide. Cimetidine inhibits uptake transporters (OCT) and efflux transporters (multidrug and toxin extrusion, MATE) responsible for renal secretion of procainamide.³³⁻³⁶ Coadministration of these drugs results in an increase in procainamide retention which can cause more pronounced side effects.

While most of the transporter research has centered on hepatic and renal transport, there is growing interest in studying other tissues as well, including the testis.^{2,26,27} Testicular transport mechanisms are especially important regarding the blood-testis barrier (BTB). The anatomical portion of the BTB, the basal tight junctions between the Sertoli cells, can impede xenobiotic diffusion between cells.³⁷⁻⁴¹ Due to the tight junctions, transepithelial transport through Sertoli cells is the primary way for a hydrophilic compound to access the seminiferous tubules at significant concentrations. Additionally, efflux transporters along the basal membrane of Sertoli cells may serve a protective function to developing sperm by preventing the germ cells from being exposed to potential toxicants.^{25,26,42,43} Uptake transporters are important for nutrients, such as lactate, nucleosides and carnitine, that cannot diffuse past the tight junctions but are needed by the germ cells.⁴⁴⁻⁴⁶ From a clinical perspective, the efflux transporters can act as obstacles for drugs requiring entry into the seminiferous tubules in order to achieve full therapeutic effect.²⁷ The transepithelial transport pathways can also be potentially problematic by providing a mechanism of access for toxicants to the male genital tract (MGT), especially if the transporters are able to concentrate their substrates inside the MGT. It's also possible for transport in the seminiferous tubules to have an effect downstream in the epididymis. Fluid in the lumen of the seminiferous tubules flows into the epididymis where up to 99% of the water is reabsorbed, resulting in a dramatic increase in concentration inside the epididymal duct, potentially causing toxicity.^{47,48} This mechanism of testicular transport followed by epididymal concentration may be occurring with drugs used to

treat HIV.²⁷ In short, a solid understanding of transport within the testis, and especially in seminiferous tubules, has the potential to impact many important fields relevant to medicine. It can improve the disposition of newer drugs to the testis, predict potential toxicants, present a better understanding of physiological (and potentially, pathological) process, and provide a mechanism for toxicity.

Signature Lesion

When the toxicologic pathologist is confronted with a testicular lesion, the sole use of histology will not determine if xenobiotic transporters are involved in the selective concentration of a toxicant in the seminiferous tubules or interstitium of the testis. Such information can only come about from a disposition study (or high-resolution whole body autoradiography) which isolates the testis into its component parts and analyzes them separately. Such conclusions could be inferred from disposition studies in other tissues that express the same transporter and are shown to impact the concentration of a select xenobiotic. Since there is not a lot of scientific literature determining the expression of transporters in the testis, there are currently little to no known examples of transporters causing toxicity in the male reproductive tract. However, this should not be misinterpreted to mean that transporters do not play a role in testis pathology and could be responsible for at least the severity of toxicity observed. This might then lead to screening for clean back-up compounds not based on their benign effects on the testis, but based on not being substrates for a particular transporter.

Organic Transporters

Organic transporters are clinically relevant due to their potential impact on drug disposition. The mRNA expression for several xenobiotic transporters of the testis, Sertoli cells and epididymal cells has been analyzed.^{1,2} Many of the xenobiotic transporters found in testis are ABC efflux transporters (typically transporting substrates out of the tubules) that have a wide variety of substrates. These include P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), MRP1, MRP4, MRP5, MRP7, and MRP8.^{2,15,16,25,26,42} All of these, except for MRP7, have been localized within the testis.^{26,42,49,50} BCRP is highly expressed on the luminal side of interstitial capillaries, and can be expressed by Sertoli cells in a stage-specific (stages VII-VIII) manner at the apical ectoplasmic specialization (ES).^{25,42,49,51} The localization of P-gp is known to be in the luminal membrane of capillaries, Leydig cells, and along the BTB, although whether it is expressed by Sertoli cells or myoid cells remains controversial.^{16,42} MRP1 and MRP4 have also been shown to be expressed by Leydig cells and on the basolateral membrane of Sertoli cells in rodents.^{25,26,42,51-53} Interestingly, MRP4 is localized to the apical membrane in humans and macaques testis.²⁶ MRP5 has only been detected in Leydig cells and MRP8 has been localized to round spermatids.²⁶ The physiological function of these

multispecific efflux transporters is primarily thought to be cytoprotective, i.e., keep potential toxicants from reaching developing sperm.^{16,25,26,54,55} However, the localizations of MRP5 and MRP8 indicate that these transporters have some other function. While there is speculation that these transporters participate in steroid (MRP5) or growth hormone (MRP8) efflux, the physiological role of these proteins within the testis remains to be determined.²⁶

In addition to ABC efflux transporters, several organic transporters from the SLC super family have been investigated in the testis. These include ENT1, ENT2, several organic anion transporting polypeptides (OATP), organic anion transporter 2 (OAT2), organic cation transporter 3 (OCT3), and OCTN2.^{1,2,27,56-58} ENT proteins are thought to be important for allowing vital nucleosides to reach the developing germ cells.⁴⁴ Additionally, these transporters have been shown to interact with some nucleoside-based drugs used for HIV treatment and chemotherapy agents indicating that these transporters may be clinically relevant. ENTs transport substrates bidirectionally based on concentration gradient.^{59,60} These characteristics are particularly interesting regarding testicular function since ENT1 is on the basolateral membrane of Sertoli cells and ENT2 is on the apical membrane.²⁷ If concentration of a substrate is high outside the seminiferous tubule, ENT1 will transport the xenobiotic into the Sertoli cell and, as intracellular concentration rises, ENT2 will transport the substrate from inside the cell to the lumen of the seminiferous tubule. This represents a transepithelial pathway by which nucleosides can translocate from the blood to the germ cells within the tubules and possibly to the epididymis.

OATPs are bidirectional transporters that interact with a wide variety of substrates, including thyroid hormones, organic dyes, bile salts, anionic oligopeptides and many other xenobiotics including clinical drugs.⁵⁷ At least 5 (Oatp1a5, -3a1, -6b1, -6c1, and -6d1) have been shown to be expressed by spermatogonia.⁵⁶ Oatp6a1 has also been found in the testis, specifically in Sertoli cells, spermatogonia, and Leydig cells, although the endogenous substrate for this protein is currently unknown.^{61,62} There has been some interest in the transport of sulfated steroids.⁶³ It is speculated that these biologically inactive compounds may become de-sulfated (and thus active) following uptake into the testis by an unknown transport mechanism.^{61,63} Sodium-dependent organic anion transporter (SOAT) was found to transport sulfated steroids but it is expressed primarily by germ cells.⁶¹ OAT2 has been found in several tissues other than the testis, most prominently in the liver and kidney.^{64,65} OAT2 transports small anionic compounds via exchange with succinate or fumarate.⁶⁴ There are many endogenous and clinically relevant drugs that are substrates for OAT2 including cGMP, nucleobases, prostaglandins, urate, methotrexate, zidovudine, pravastatin and cimetidine. The localization for OAT2 in the testis is currently unknown. There is also evidence that organic cation transporters (OCT) 1 and 3 are expressed by Sertoli cells on the basal and apicolateral membranes respectively.^{2,66}

It has been known for quite some time that L-carnitine within the MGT is important for male fertility. Concentrations of L-

carnitine within the epididymis is 1000 fold higher than plasma concentrations.^{45,67-71} L-carnitine aids in the fatty acid oxidation and has systemic antioxidant activity. It has been speculated that L-carnitine is beneficial for fertility by supplying maturing sperm with energy through increase in lactate dehydrogenase C₄ (LDH-C₄), stabilizing plasma membranes, and protecting sperm from reactive oxygen species.^{45,46,67,71} Since the BTB would prevent L-carnitine from passively diffusing into the seminiferous tubules, this nutrient must be transported through Sertoli cells. One L-carnitine transporter known to be expressed by Sertoli cells is OCTN2, a multispecific uptake transporter that requires sodium.^{1,2,71} In addition to carnitine, OCTN2 has also been shown to transport clinical drugs such as verapamil and quinine.^{67,71,72} Other L-carnitine sodium-independent transporters like OCTN3 and carnitine transporter 2 (CT2) have also been shown to be expressed in mouse and human testis respectively and likely participate in carnitine uptake as well.^{68,72}

It is not surprising that glucose metabolism is known to be critical for proper sperm maturation.⁷³ One of the functions of the Sertoli cells is to import glucose from the blood and metabolize it to lactate which is the primary energy source of the germ cells. This requires glucose gaining entry into the Sertoli cells via the glucose transporter (GLUT). In the context of testis, glucose transport is accomplished by GLUT1, GLUT3, and GLUT8.⁷³ GLUT1 and GLUT3 have been shown to be sensitive to regulation from hormones and growth factors and have been localized to endothelial cells, basal membrane of Sertoli cells, and myoid cells.⁷⁴

Inorganic Transporters

Some inorganic transporters of the testis have been studied, typically with the goal of establishing the physiological processes of the testes. Water and closely related ion transport is also important for proper fertility.⁷⁵⁻⁷⁷ Water is crucial for sperm to move from the lumen of the seminiferous tubules all the way to the vas deferens (ductus deferens).⁷⁸ Water is able to penetrate the BTB due to Sertoli cell expression of specialized water transporters known as aquaporins (AQP). AQP expression can change depending on the stages of the Sertoli cells, however, AQP0, 7, 8, and 11 have been shown to be expressed in seminiferous tubules.⁷⁸ Additionally, germ cells express AQP8 and 7.^{76,78} Water reabsorption in the epididymis is also important for fertility as vast amounts of water (90-99%) are removed from the efferent ducts and epididymal duct.^{47,79,80} (also see Hess, this issue). This water reabsorption is required for proper sperm development.⁷⁶ Within the epididymis, AQP1, 9, and 10 are known to be expressed and sodium hydrogen exchanger 3 (NHE3) is also known to be important for water reabsorption and maintenance of luminal pH.^{75,77} If water reabsorption is disturbed (increased or decreased), significant damage can be produced in the testis (see Hess, this issue).

Many of these inorganic transporters aid in the physiological processes of the testis, but they may also contribute to toxicity. Examples include transporters that allow the entry of metal ions

Figure 1. A representation of the testis summarizing the general locations of known transporters and their directionality. *indicates stage specific localization of transporter. **indicates controversial localization.

into Sertoli cells. These can be especially problematic since several metals, such as cadmium (Cd) and arsenic (As) are known to be reproductive toxicants.⁸¹ The primary transporter known for Cd, manganese (Mn) and zinc (Zn) for Sertoli cells is Zrt-, Irt-related protein 8 (ZIP8).^{81,82} Iron (Fe) is an essential nutrient for developing sperm but can easily reach cytotoxic concentrations if not carefully regulated.^{83,84} The main transporters involved in Fe

transport for germ cells are transferrin receptor (TfR1) and divalent metal transporter 1 (DMT1).⁸³ Small amounts of TfR1 located at the basolateral membrane of Sertoli cells are likely responsible for Fe uptake. TfR1 is also expressed by primary spermatocytes indicating that it is important for early germ cell development. DMT1 is localized to the apical membrane near elongating spermatids which suggests that it is vital for Fe transport later in sperm development.⁸³

While many other body systems (liver and kidney) have classically been the focal point of transporter research, the testis is gaining attention due to the importance of the BTB on drug⁶¹ disposition and interests in fertility. The testis, especially Sertoli cells, express a complex array of transporters that perform various functions and potentially have several clinical effects. **Figure 1** summarizes the transporter localization and directionality within the testis discussed in this review. Research regarding the impact transporters on the testis is still in its early stages. Further understanding of transport processes of the testis can provide a basis for the physiology of the testis which can help elucidate pathology in diseased tissue.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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