Transepithelial Transport Across the Blood-Testis Barrier

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Abstract

The blood-testis barrier protects developing germ cells by limiting the entry of xenobiotics into the adluminal compartment. There is strong evidence that the male genital tract can serve as a sanctuary site, an area of the body where tumors or viruses are able to survive treatments because most drugs are unable to reach therapeutic concentrations. Recent work has classified the expression and localization of endogenous transporters in the male genital tract as well as the discovery of a transepithelial transport pathway as the molecular mechanism by which nucleoside analogs may be able to circumvent the blood-testis barrier. Designing drug therapies that utilize transepithelial transport pathways may improve drug disposition to this sanctuary site. Strategies that improve disposition into the male genital tract could reduce the rate of testicular relapse, decrease viral load in semen, and improve therapeutic strategies for male fertility.

Introduction

The endogenous function of the blood-testis barrier (BTB) is to protect developing germ cells, provide an immune privileged environment for meiosis and normal male fertility maintenance (Fijak & Meinhardt 2006, Lin *et al.* 2012, Mruk & Cheng 2015, Stanton 2016). The BTB contributes to normal male fertility by preventing the production of antibodies against sperm and leading to male infertility, as well as limiting the entry of harmful compounds (Francavilla *et al.* 2007, Mital *et al.* 2011, Mruk *et al.* 2011). The anatomical portion of the BTB is composed of tight junctions between epithelial cells of the seminiferous tubule (Sertoli cells) (Kato *et al.* 2005, Mital *et al.* 2011, Mruk & Cheng 2015). Tight junctions and polarized epithelial cells limit the entry of hydrophilic compounds. The physiological components of the blood-testis barrier include efflux transporters such as P-glycoprotein (P-gp) and ion channels (Fromm 2004, Mital *et al.* 2011, Mruk *et al.* 2011). Efflux transporters present on the basal membrane of Sertoli cells may remove any potentially harmful compounds that successfully enter Sertoli cells (Figure 1) (Mital *et al.* 2011, Mruk *et al.* 2011). Therefore, the presence of the BTB poses a unique clinical challenge in the treatment of several diseases including cancers, viral infections, and the development of therapeutic strategies for male fertility (Byrn & Kiessling 1998, Kiessling *et al.* 1998, Kulkarni *et al.* 2010, Cheng & Mruk 2012). The BTB may serve as a sanctuary

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site, an area of the body where cancers or viruses are able to survive typical treatments (Eilers *et al.* 2008, Ronaldson *et al.* 2008, Dahl *et al.* 2010, Palmer *et al.* 2011). Other regions of the male genital tract (MGT) may also participate in sanctuary functions. The proximal end of the MGT is at the seminiferous tubules in the testis and ends at the urethra (Mawhinney & Mariotti 2012). The seminiferous tubules are the site of spermatogenesis and the tubules converge at the rete testis (Mawhinney & Mariotti 2012). Sperm migrate through the seminiferous tubules, the rete testis, the caput, corpus and cauda (sperm maturation and storage site) of the epididymis, and exit during ejaculation through the vas deferens (Mawhinney & Mariotti 2012).

Certain cancer and viral therapies that are unable to enter the MGT may at least partially explain why relapses occur. HIV can be detected in the seminal plasma of patients who have an undetectable viral load in blood serum (Le Tortorec & Dejuq-Rainsford 2010). Cancer cells can also be detected in the testes of patients who have undergone treatment when undetectable in the rest of the body (Grundy *et al.* 1997, Arya *et al.* 2010, Locatelli *et al.* 2012, de Góes *et al.* 2014). Tight junctions, efflux transporters and endogenous transepithelial transport pathways are not only obstacles for cancer and antiviral therapies, they are obstacles for the development of male contraceptives and male fertility treatments (Cheng & Mruk 2012). Discovering methods to improve drug disposition into the MGT will have a substantial impact on cancer and viral therapies as well as therapeutic strategies for male fertility. In this review, we will discuss the impact of the BTB on effective drug disposition and how endogenous transepithelial transport pathways may improve it.

Blood-Testis Barrier-Structure and Function

The testis is a dynamic organ that possesses numerous cell types to aid in the development of sperm cells (spermatogenesis) or androgen production (steroidogenesis). The seminiferous tubule is the site of spermatogenesis within the testis. Leydig cells are interstitial cells (i.e. in between the seminiferous tubules) that generate testosterone following stimulation by LH. Myoid cells are contractile cells that surround the seminiferous tubule and help with the movement of spermatozoa from the seminiferous tubule and into the epididymis (Palombi *et al.* 2002, Mruk & Cheng 2015). However, the true BTB begins with Sertoli cells. Sertoli cells are the epithelial cells within seminiferous tubules that provide functional support and many nutrients including nucleosides during spermatogenesis (Kato *et al.* 2005, Mruk & Cheng 2015). Adjacent Sertoli cells form tight junctions, which form the anatomical component of the BTB (Cheng & Mruk 2004, Mruk & Cheng 2015, Mital *et al.* 2011, Stanton *et al.* 2016). Tight junctions contribute to the permeability and selectivity of epithelial barriers (Powell, 1981, Schneeberger & Lynch, 2004). The presence of tight junctions contributes to protecting developing germ cells in addition to providing an immune privileged site for meiosis (Fijak & Meinhardt 2006, Mruk & Cheng 2015). Without the presence of the BTB, androgens and testicular dendritic cells, developing germ cells would be recognized as foreign and be under autoimmune attack (Fijak & Meinhardt 2006). Androgens play a role in the inhibition of proinflammatory cytokines expression and testicular dendritic cells regulate antigen-specific immunity and T-lymphocyte activation (Fijak & Meinhardt 2006). These factors highlight the complex functions of the testis. Although developing germ cells are protected, endogenous compounds required for

spermatogenesis must be able to bypass the BTB. This may be possible by using transepithelial transport pathways produced by the Sertoli cells.

Transporters in the Male Genital Tract (MGT)

Transport of drugs across a biological membrane by transporters is often required for drugs to reach their targets efficiently. Transporters have a substantial impact on the disposition and efficacy of xenobiotics and their presence in the MGT (Robillard *et al.* 2012, Fietz *et al.* 2013, Klein *et al.* 2014ab, Klein *et al.* 2015, Huang *et al.* 2016, Klein *et al.* 2017). Simple diffusion, facilitated transport, and secondary active transport move molecules down their electrochemical gradient and do not require energy. The solute carrier transporter (SLCs) family includes both facilitative transporters and secondary active transporters that do not require energy (ATP) to function. The other family of membrane transporters is the ATP-binding cassette transporters (ABCs) which are primary active transporters. These transporters utilize ATP to translocate molecules against an electrochemical gradient. Several transporters have been shown to play a role in the disposition of chemotherapeutics and antiretroviral therapies (Jeha *et al.* 2004, Klein *et al.* 2013, Huang *et al.* 2016, Macanas-Pirard *et al.* 2017).

Even with the presence of a physiological barrier, it is well known that toxicants that target developing germ cells must circumvent or disrupt the BTB. Examples of male reproductive toxicants include Cisplatin, Lead(II) (Pb^{2+}), Cadmium (II) (Cd^{2+}), and NRTIs. Cisplatin is a male reproductive toxicant commonly used to treat testicular tumors and many patients receiving this chemotherapeutic have temporary or permanent azoospermia, which is the absence of motile sperm in semen (Colpi *et al.* 2004). Transporters that are known to facilitate the movement of cisplatin across cell membranes include copper transporter 1 and 2 (CTR1 CTR2), P-type copper transporting ATPase 7A and 7B (ATP7A and ATP7B), organic cation transporter 2 (OCT2), and multidrug extrusion transporter 1 (MATE1) (Ciarimboli, 2014). Lead (Pb^{2+}) and cadmium (Cd^{2+}) are occupational and environmental toxicants that are detectable in seminal plasma and may be linked to infertility (Benoff *et al.* 2000, Wijesekara *et al.* 2015). These ions are known to be transported by divalent metal transporter 1 (DMT1) (Bressler *et al.* 2004). Arsenic is another toxicant that is detectable in seminal plasma and is associated with reduced semen quality (Xu *et al.* 2012, Wang *et al.* 2016).

Like these heavy metals, the NRTIs AZT, didanosine, tenofovir, and lamivudine are detectable in seminal plasma of HIV-1 patients on antiretroviral therapy (Pereira *et al.* 1999, Lowe *et al.* 2007). Nucleoside transport in humans is mediated by concentrative and equilibrative nucleoside transporters (CNTs, ENTs). Uptake of these compounds in Sertoli cells is mediated by ENT1 (Klein *et al.* 2013). Passage through Sertoli cells into the adluminal compartment is possible through transepithelial transport pathways created by basal membrane uptake transporters and apicolateral membrane efflux transporters (Klein *et al.* 2013). A mechanism by which Sertoli cells protect developing germ cells is through the expression of efflux transporters on the basal membrane (Bart *et al.* 2004, Klein *et al.* 2014b). This is thought to reduce the level of toxicants within the seminiferous tubules. However, this can also prevent therapeutic compounds from crossing the BTB. The

importance of studying transporters in the BTB has been recognized, and information regarding the expression and localization of important SLCs and ABCs in the MGT will be described along with their implications in disease.

Solute Carrier Transporters (SLCs)—Several SLCs are expressed in the testis (Fietz *et al.* 2013, Klein *et al.* 2013, Klein *et al.* 2015, Huang *et al.* 2016). One example is organic anion transporting polypeptides (OATPs), which are bidirectional transporters with of a wide variety of substrates including endogenous amphipathic compounds, xenobiotics and pharmacological drugs (Su *et al.* 2011, Roth *et al.* 2012). OATP1B1 and OATP2B1 are localized at the seminiferous epithelium and throughout the testicular interstitium in the human testis (Huang *et al.* 2016). OATP6A1 is predominantly expressed in the testis and is present in human Sertoli cells, Leydig cells and spermatogonia (Roth *et al.* 2012, Fietz *et al.* 2013, Klein *et al.* 2015). Organic anion transporters (OATS) are responsible for the transport of steroid hormones, biogenic amines, and many drugs including the NRTI tenofovir (Roth *et al.* 2012). OAT1 is expressed in the testicular endothelium (Huang *et al.* 2016).

The uptake transporters organic cation transporters 1 and 3 (OCT1, OCT3) are present in Sertoli cells. OCT1 is localized to the basal membrane and OCT3 is localized to the apicolateral membrane (Maeda *et al.* 2007, Klein *et al.* 2015). Carnitine transporters are also expressed in the testis. Carnitine is essential to fatty acid oxidation and subsequent energy production necessary for spermatogenesis. OCTN2 is expressed on the basal membrane and OCTN3 is on the apicolateral membrane of Sertoli cells (Kobayashi *et al.* 2005, Klein *et al.* 2015). OCTN2 has been found to mediate carnitine uptake in rat Sertoli cells (Kobayashi *et al.* 2005). The equilibrative nucleoside transporters ENT1 and ENT2 are also present in Sertoli cells and ENT1 is present on the basal membrane of human Sertoli cells, and ENT2 is present on the apicolateral membrane of human Sertoli cells (Kato *et al.* 2005, Klein *et al.* 2013, Klein *et al.* 2015, Huang *et al.* 2016). As previously mentioned, chemoresistance to cytarabine can occur when ENT1 activity is decreased or the transporter is removed from the cell surface (Macanas-Pirard *et al.* 2017). This is why it is important to examine whether or not the expression or localization of transporters changes with disease state, as it may impact the disposition of drugs that require these transporters to reach their therapeutic target. This was investigated in HIV positive patients. mRNA and protein expression of OATP1B1, OATP2B1, OAT1, CNT1 and ENT2 in HIV infected and uninfected testicular tissue was detected and the localization of these SLCs was the same in both groups (Huang *et al.* 2016). Further investigation of changes in SLC transporter expression and localization in other testicular disease states is needed and can be used to guide treatment plans. However, the strong evidence in support of the presence of transporters in the testis demonstrate that substrates for these transporters may be able to circumvent the BTB for therapeutic benefit. Many drugs are substrates for these transporters and their expression, activity and localization should be considered during the drug development process.

ATP-Binding Cassette Transporters (ABCs)—The ABC family includes broad specificity efflux transporters that contribute to the protective function of the BTB such as P-gp, breast cancer resistance protein (BCRP), and multidrug resistance-associated proteins (MRPs). P-gp, BCRP, MRP1, MRP4, MRP5, and MRP8 are expressed in normal testicular

tissue. P-gp is present throughout the seminiferous epithelium, interstitial space and within Leydig cells (Bart *et al.* 2004, Su *et al.* 2011, Huang *et al.* 2016, Klein *et al.* 2015). BCRP is localized to the basal side of the seminiferous epithelium (Bart *et al.* 2004, Klein *et al.* 2015, Huang *et al.* 2016) and MRP1 is expressed by Leydig cells and the basal membrane of Sertoli cells (Bart *et al.* 2004, Klein *et al.* 2014b). In human and monkey, MRP4 is localized to the basal membrane of Sertoli cells, but on the apicolateral membrane in mature and immature rats (Klein *et al.* 2014b, Huang *et al.* 2016). MRP5 is present in Leydig cells and MRP8 is present in round spermatids (Klein *et al.* 2014b).

Protein and mRNA expression of P-gp, BCRP, MRP1, MRP4, and was detected and expressed at similar levels in both uninfected and HIV infected testicular tissue (Huang *et al.* 2016). Their localization remains unchanged in both HIV infected and uninfected tissues. However, this is not the case in testicular tumors where ABC expression depends on tumor type. Despite variability between individuals, one study showed seminomas expressed P-gp, BCRP or MRP1 while testicular lymphomas typically showed strong P-gp or BCRP expression and little MRP1 expression (Bart *et al.* 2004). In both seminomas and testicular lymphomas, MRP2 was weakly expressed (Bart *et al.* 2004). These data demonstrate the importance of examining differences in expression and localization of ABC transporters in different disease states. Chemotherapies, antivirals, fertility treatments and male contraceptives that are substrates for efflux transporters that have altered expression or localization in testicular disease states may have implications in variable drug response.

Transepithelial Transport and its Implications for Chemotherapy, Antiviral Therapy, Male Fertility and Contraception

Transepithelial transport through Sertoli cells by basal membrane uptake transporters and apicolateral membrane efflux transporters provides a pathway for endogenous substrates and drugs to cross the BTB (Figure 2). One classic method of studying transepithelial transport pathways is through the culture of primary Sertoli cells on extracellular matrix coated transwell inserts (Kato *et al.* 2005, Mruk & Cheng 2011, Klein *et al.* 2013). Endogenous nucleosides, like uridine, have been used to determine possible transport mechanisms of NRTIs. NRTIs have similar structures to endogenous nucleosides which are known to be transported by ENTs. Uridine uptake in Sertoli cells is mediated by ENT1 and efflux is mediated by ENT2 (Kato *et al.* 2005, Klein *et al.* 2013). Based on transport data in primary Sertoli cells, a transepithelial pathway has been established. Using this same model, the NRTIs AZT, didanosine and tenofovir inhibited basal uridine uptake, suggesting that these compounds may utilize the same basal membrane uptake pathway as uridine, but the identification of apicolateral membrane efflux transporters responsible for their entry into the adluminal compartment remains unknown (Klein *et al.* 2013). Transepithelial transport of nucleosides through Sertoli cells by ENT1 and ENT2 is one of many potential pathways that can be utilized to improve drug disposition.

Cancer and Chemotherapy by Transepithelial Transport

Testicular relapse is the recurrence of cancer in the testis after initial treatment or period of improvement (Arya *et al.* 2010, Kulkarni *et al.* 2010, Locatelli *et al.* 2012). This can occur with testicular cancers such as seminomas or some types of leukemia such as acute

lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML) (Dave *et al.* 2007, Arya *et al.* 2010, Kulkarni *et al.* 2010, de Góes *et al.* 2014, Hanna & Einhorn 2014). Both bilateral orchiectomy (removal of both testes), testicular irradiation, and chemotherapy are courses of treatment for relapsed testicular cancers and leukemia (Arya *et al.* 2010, Kulkarni *et al.* 2010, Hanna & Einhorn 2014). Orchiectomy and testicular irradiation cause infertility and reduced testosterone production which pose major quality of life issues, especially in young patients (Locatelli *et al.* 2012). The incidence of testicular relapse varies with the type and stage of testicular cancer, but has been reported to occur in nearly 20% in nonseminoma tumors and 13% in seminoma tumors (Hanna & Einhorn 2014, Kollmannsberger *et al.* 2015)

The incidence of testicular relapse of ALL in children is around 10–20% (Dave *et al.* 2007, Arya *et al.* 2010, Kulkarni *et al.* 2010). Nucleoside analogs such as cytarabine, clofarabine, and nelarabine have demonstrated their effectiveness as chemotherapeutics used to treat leukemia such as ALL (Jeha *et al.* 2004, www.cancer.gov/about-cancer/treatment/drugs April 28, 2018). To successfully eliminate tumor cells that have penetrated the BTB, chemotherapy treatments must achieve sufficiently high concentrations within the BTB. Transporters play a large role in this process. Alterations to transporter function/expression may alter drug exposure and subsequently cause adverse drug reactions or insufficient therapeutic levels of the drug. This has been observed with the nucleoside analog cytarabine. Cytarabine and other nucleoside based chemotherapies require equilibrative nucleoside transporter 1 (ENT1) to elicit their effect, and in primary human leukemia cells, chemoresistance is mediated by bone marrow stromal cell release of soluble factors that is associated with a decrease in activity or removal of ENT1 from the leukemia cell surface (Macanas-Pirard *et al.* 2017). Additionally, in a meta-analysis of pancreatic cancer patients treated with the nucleoside analog gemcitabine, high ENT1 expression in tumors was associated with improved overall survival and longer disease free survival (Zhu *et al.* 2014). These data highlight the importance of transporter function in successful cancer treatment. Since transporters are critical for hydrophilic compounds to access the testis, a better understanding of the transport mechanisms by which chemotherapies enter the testis is needed especially in regard to testicular relapse. Uncovering these pathways could improve chemotherapy disposition to the testis and lead to an increase in the effectiveness of these therapies, improve prognoses, and reduce the rate of testicular relapse.

Virus Transmission and Antiviral Therapy by Transepithelial Transport

The MGT is a particularly important site for research on viruses that are sexually transmitted such as human immunodeficiency virus (HIV). Nearly 75% of HIV infected patients in the US are men and transmission from men occurs with higher efficiency than from women (Royce *et al.* 1997, www.cdc.gov/hiv/library/reports/hiv-surveillance.html April 28, 2018). Free HIV particles have been detected in seminal plasma even when undetectable in blood plasma (Kiessling *et al.* 1998, Zhang *et al.* 1998, Le Tortorec & Dejucq-Rainsford 2010) and it has been demonstrated that the testis is capable of HIV infection *ex vivo* (Roulet *et al.* 2006). In addition, it is well established that HIV strains persist in semen and evolve separately from strains in the blood (Le Tortorec & Dejucq-Rainsford 2010). Infected cells of the MGT are a critical target for therapy since this viral population is often transmitted and there is little information known regarding drug distribution to these sites of infection.

One study detected HIV in germ cells of infected patients indicating germ cells could potentially be a viral reservoir beyond the BTB (Shevchuk *et al.* 1998). Current HIV/AIDS treatments include nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) such as didanosine, zidovudine (AZT), tenofovir, and lamivudine and have been shown to be very effective at reducing viral load. NRTIs target the HIV replicative life cycle and can reduce the viral load in plasma, improve overall immune function, prolong survival, reduce HIV associated morbidity, improve quality of life, and reduce the risk of transmission of HIV to others (Pau & George, 2014, Looney *et al.* 2015). HIV-1 patients being treated with didanosine and tenofovir had detectable amounts of drug in seminal plasma, indicating that these drugs are capable of entering the MGT (Lowe *et al.* 2007).

Other viruses are also detectable in seminal plasma indicating penetration into the MGT. Herpes simplex virus (HSV) is a sexually transmitted disease that has been detected in semen (Monavari *et al.* 2013, Gimenes *et al.* 2014). Current treatments include the nucleoside analogs acyclovir, famciclovir and valaciclovir (Vere Hodge & Field 2013). Acyclovir has been detected in semen, indicating that this nucleoside analog is capable of entering the MGT (Douglas Jr *et al.* 1988). All of these studies strongly support the notion that the MGT is a sanctuary site that is capable of viral replication independent from the blood. Discovering the mechanistic pathways by which these compounds and other nucleoside analogs enter the MGT may provide insight to improve drug disposition into the MGT, successfully eliminate a sanctuary site, and decrease the likelihood of viral transmission from semen.

Fertility and Male Contraception-Potential to use Transepithelial Transport Pathways

Identifying transepithelial transport pathways in which chemotherapeutic and antiviral drug disposition can be improved may also impact therapeutic strategies for male fertility. There is great interest in controlling male fertility for both treating infertility and contraception. It is estimated that 30% of cases of male infertility are of unknown etiology which emphasizes the importance of research of basic mechanisms in male reproductive physiology (Gudeloglu *et al.* 2014). Even when the cause is known, there are limited treatment options (Palermo *et al.* 2014) These treatments include hormones to improve endocrine dysregulation, surgery to correct enlarged scrotal veins (varicocele) or to remove obstructions, antibiotics for infections and assisted reproductive technology such as in vitro fertilization or intracytoplasmic sperm injection (Palermo *et al.* 2014). One study demonstrated that prednisone (a corticosteroid) is capable of improving sperm count and motility in oligozoospermic (low concentration of sperm) patients (Milardi *et al.* 2017).

One of the known obstacles in creating a male contraceptive and infertility treatments has been the BTB (Palombi *et al.* 2002, Su *et al.* 2010, Qian *et al.* 2013). Sperm motility, sperm formation, and maturation in the testis and epididymis represent promising targets for developing male contraceptives and infertility treatments (Chao *et al.* 2014, Gudeloglu *et al.* 2014, Palermo *et al.* 2014). A variety of hormonal contraceptives including testosterone-based approaches and gonadotropin-releasing hormone (GnRH) antagonists and several non-hormonal contraceptives have shown promise as male contraceptives (Cheng & Mruk 2012, Chao *et al.* 2014). These include testosterone alone, testosterone-progestin combinations,

and testosterone-GnRH combinations (Cheng & Mruk 2012, Chao *et al.* 2014). Although suppressing gonadotropins can suppress spermatogenesis in most men, it does not work for all men (Chao *et al.* 2014). The continuing challenges in developing male contraceptives include ensuring that they are completely reversible with no negative long-term side effects (Chao *et al.* 2014).

Adjudin is a non-hormonal contraceptive that was found to reversibly disrupt germ cell adhesion but does not disrupt BTB integrity (Su *et al.* 2010). It was also found to bind and downregulate an efflux transporter, breast cancer resistance protein (BCRP) in the testis (Qian *et al.* 2013). Improved analogs of adjudin could potentially downregulate other efflux transporters to improve their disposition to the seminiferous tubules. In addition to downregulating efflux transporters, drug targeting to the seminiferous tubules could utilize the same mechanistic pathways that endogenous substrates such as nucleosides use to cross the BTB. Acyline is a GnRH antagonist that has also shown promise as a male contraceptive (Herbst *et al.* 2002, Amory *et al.* 2009, Cheng & Mruk 2012). In humans, it is a potent suppressor of testosterone and gonadotropins such as luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Herbst *et al.* 2002, Amory *et al.* 2009). More research is needed on the long term effects of acyline but both adjudin and acyline demonstrate the progress that has been made in developing male contraceptives. There are many potential targets for infertility treatments and male contraceptives that will require more extensive knowledge of the selectivity of the BTB before therapies can be designed and developed.

Summary and Conclusions

The inability of injected dyes to penetrate the testis lead to the discovery and research on the anatomical and physiological components of the blood-testis barrier (Kormano, 1967). Since this discovery, uncovering endogenous pathways in which both endogenous and exogenous compounds are able to circumvent the blood-testis barrier has become of great interest. The effectiveness of current chemotherapies, antivirals, and male contraceptives depends on the compound's ability to circumvent the BTB whether it be by diffusion (common for hydrophobic compounds), or through passive or active transport. The physiological role of the BTB is to provide a secure environment for spermatogenesis which results in limited accessibility for endogenous compounds and substrates. Transporters have a major impact on drug disposition and efficacy, therefore it is important for drug development to understand the full complement of transporters that may influence disposition across the BTB. Determining the localization of other transporters and mapping their coordinated transport could provide a mechanistic rationale for directed drug development to overcome the unique challenges that the BTB poses.

Comparing and contrasting the characteristics of a broad library of compounds that are able to bypass the BTB may provide insight on what chemical traits and groups are essential for transepithelial transport through Sertoli cells to circumvent the BTB. This information can be used to generate a predictive model that will enable more informed drug design. Once created, a predictive model would allow researchers to screen thousands of compounds efficiently. Nucleosides and some nucleoside analog drugs are known to penetrate the BTB (Douglas Jr *et al.* 1988, Lowe *et al.* 2007). Since there is a known transepithelial transport

pathway that these compounds may use, a predictive model can be developed based on a relatively low throughput screen of nucleoside based compounds that utilize the ENT1/ENT2 transepithelial transport pathway. Once other transepithelial transport pathways are discovered, additional predictive models can be generated to create a more complete picture of transepithelial transport across the BTB. This will ultimately inform the drug design of chemotherapies, antivirals, fertility drugs, and male contraceptives which can be expected to improve patient outcomes by decreasing the likelihood of testicular relapse, viral transmission from semen, as well as advance the development of compounds for male fertility. Investigating the transepithelial pathways of compounds already known to cross the BTB through Sertoli cells, such as NRTIs, provides an excellent starting point for understanding the mechanisms of circumventing the BTB and thereby improve treatment for a variety of patients.

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Figure 1:
Physiological Function of the Blood-Testis Barrier

Figure 2:
Transepithelial Transport Across the Blood-Testis Barrier