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Xenobiotic transporter expression along the male genital tract☆

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ABSTRACT

The male genital tract plays an important role in protecting sperm by forming a distinct compartment separate from the body which limits exposure to potentially toxic substrates. Transporters along this tract can influence the distribution of xenobiotics into the male genital tract through efflux back into the blood or facilitating the accumulation of toxicants. The aim of this study was to quantitatively determine the constitutive mRNA expression of 30 xenobiotic transporters in caput and cauda regions of the epididymis, vas deferens, prostate, and seminal vesicles from adult Sprague–Dawley rats. The epididymis was found to express at least moderate levels of 18 transporters, vas deferens 15, seminal vesicles 23, and prostate 18. Constitutive expression of these xenobiotic transporters in the male genital tract may provide insight into the xenobiotics that can potentially be transported into these tissues and may provide the molecular mechanism for site specific toxicity of select agents.

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1. Introduction

In order for a xenobiotic compound to influence function of a target tissue, it must be present at sufficient concentrations. Transporters are one of the primary determinants of xenobiotic absorption, distribution, and elimination processes by facilitating uptake into or efflux from cells [1]. Several families of proteins have been demonstrated to transport a wide variety of clinical drugs and endogenous substrates. These transporters include multiple drug resistance (Mdr) proteins, multidrug resistance-associated proteins (Mrp), organic anion transporters (Oat), organic anion transporting polypeptides (Oatp), organic cation transporters (Oct), equilibrative nucleoside transporters (Ent) and concentrative nucleoside transporters (Cnt). These transporters have broad and frequently overlapping substrate specificities that include many clinically used drugs in addition to environmental toxicants [2–4]. Xenobiotic transporters may play an important part in excluding these compounds from tissues, or conversely facilitating the distribution and tissue-specific accumulation of select agents. While drug transport has been well studied in organs such as the kidney, intestine, and liver, there is a paucity of information concerning transporter expression in the male reproductive system [3,5,6]. This leaves a significant gap in our knowledge concerning the categories of compounds that are excluded from or are able to accumulate within the male reproductive system.

The male genital tract (MGT) begins with the seminiferous tubules located inside the testis and are the site at which spermatogenesis occurs. These tubules converge at the rete testis before connecting to the proximal region of the epididymis (caput). The spermatids mature as they move through the epididymis and become fertile in the distal section of the epididymis (cauda) where they are stored until ejaculation. During ejaculation, the sperm travels through the vas deferens (ductus deferens) while the seminal vesicles and prostate add secretions to the tract which become the primary components of seminal plasma.

One of the functions of the MGT is to limit sperm exposure to potentially toxic agents. This function is especially important in the epididymis, where the sperm are stored, and the vas deferens which serves as the route for sperm during ejaculation. However, since sperm become heavily exposed to the secretions from the seminal vesicles and the prostate during ejaculation, any toxicants entering from these tissues could also potentially have an adverse affect sperm, as well as the sexual partners. Surprisingly, little is known concerning the expression of xenobiotic transporters in the male reproductive system. Since transporters play a pivotal role in determining the distribution of xenobiotic compounds, knowledge of tissue-specific expression of xenobiotic transporters along the MGT is essential for understanding the distribution of toxicants within the MGT. The constitutive expression levels of drug transporters in the tissues of the MGT may also provide insight into how well

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therapeutic agents are able to penetrate the various tissues of the MGT for the purposes of treating diseases, such as cancer or HIV infection [7,8]. While studies have been performed determining the expression of the major xenobiotic transporters in the blood–testis barrier and Mdr1b in the epididymis, a comprehensive analysis of transporter expression for other tissues in the MGT is currently lacking [1,9]. Therefore, this study was undertaken to determine the constitutive mRNA expression levels of 30 xenobiotic transporters in rat caput and cauda regions of the epididymis, seminal vesicles, vas deferens, and prostate in relation to the liver and kidney. Determining the mRNA expression through branched DNA analysis offers a unique advantage of being able to compare the expression profile of specific transporters with sensitivity comparable to RT-PCR across various tissue samples which is ideal for our interests. Protein analysis of these xenobiotics transporters is unfeasible to perform for a comprehensive study on this scale due to the reliance on specific antibodies which are unavailable for many xenobiotic transporters. Many xenobiotic transporters have overlapping substrate specificities making functional studies unable to discriminate between many of the transporters of interest and therefore impractical for determining expression of xenobiotic transporters in native tissue. For these reasons, studies using mRNA analysis are commonly used and seminal for understanding the transport capabilities in tissues for which little is known [5,10,11]. It is known that quantitative mRNA levels do not always match protein levels, but mRNA can generally correlate to protein levels. A complete lack of mRNA would indicate no protein present since mRNA is required to translate protein. High mRNA levels would very likely represent at least some protein expression or else the cell would be wasting energy to transcribe unused mRNA. The data presented is foundational regarding the ability of toxicants and drugs to access a variety of tissues in the MGT. An understanding of xenobiotics transport in the male reproductive system is anticipated to serve a variety of interests relevant to male reproductive biology

2. Materials and methods

2.1. Materials

Quantigene HV Signal Amplification Kit and Quantigene Discovery Kit were purchased from Genospectra (Fremont, CA). RNAzol B reagent was purchased from Tel-Test, Inc. (Friendswood, TX). All other reagents were purchased from standard scientific suppliers at the highest available purity.

2.2. Sample collection

Samples were collected from euthanized rats at least 10 weeks old. Sections from the ventral prostate were used for prostate analysis, Protocols for obtaining samples were approved by the University of Arizona Institutional Animal Care and Use Committee (IACUC).

2.3. Development of specific oligonucleotide probe sets

The probe sets used in this study were described in the following publications: Mdr1a and 1b [10]; Mdr2 [11]; Mrp1, 2, and 3 [12]; Mrp4, 5, and 6 [11]; Mrp7 and 9 [1] Oatp1, 2, 3, 4, and 5 [13]; Oct1, 2, 3, N1, and N2 [14]; Oat1, 2, and 3 [15]; Cnt1 and 2 as well as Ent1 and 2 [11]; and Cnt3 [16].

2.4. Total RNA isolation

Total RNA was isolated from 4 Sprague–Dawley rats using RNAzol B reagent as per manufacturer's protocol. RNA was pooled and used for all bDNA experiments. Each RNA pellet was resuspended in 0.2 ml of 10 mM Tris–HCl buffer, pH 8.0. The concentration of total RNA in each sample was quantified spectrophotometrically at 260 nm. RNA integrity and quality were analyzed by formaldehyde agarose gel electrophoresis with ethidium bromide staining. The quality of RNA samples was determined by the integrity and relative ratio of 28S and 18S rRNA bands.

2.5. Branched DNA assay

Specific oligonucleotide probes for each gene were diluted in lysis buffer. Substrate solution, lysis buffer, capture hybridization buffer, amplifier, and label probe buffer used in the analysis were all obtained from the Quantigene Discovery Kit. The assay was performed in 96-well format with 10 μg of RNA isolated from various tissues and then added to the capture hybridization buffer and 50 μl of the diluted probe set. The total RNA was then allowed to hybridize to the probe set overnight at 53 °C. Hybridization steps were performed per the manufacturer's protocol the following day. Luminescence of the samples was measured with a Quantiplex 320 bDNA luminometer interfaced with Quantiplex Data Management Software, version 5.02 purchased from Bayer (Walpole, MA). Background for each transporter probe set was determined using negative control wells which had all reagents except for RNA. The background was then subtracted to demonstrate expression above background levels.

3. Results

The mRNA expression levels of the major xenobiotic transporters in liver and kidney were compared to that of tissues in the male reproductive system; namely the caput and cauda regions of the epididymis, seminal vesicles, prostate, and vas deferens (ductus deferens). Expression levels for the liver and kidney were used as positive controls since the xenobiotic transporters investigated are expressed by at least one of these tissues [1]. For each transporter, either the kidney or the liver was considered the positive control depending on which tissue exhibited higher expression levels. The mRNA expression levels for the tissues along the MGT were normalized to the control tissue. Any expression that was 90% of control or higher was considered highly expressed, while 20% or higher was considered moderate expression and anything below 20% was considered low expression. Fig. 1 shows that Mdr1a, 1b (P-glycoprotein or P-gp) and Bcrp expression was high in both sections of the epididymis (96–174%). The prostate showed moderate expression levels for all three transporters while seminal vesicles only showed modest levels of Bcrp (24%). Expression of Mdr2 mRNA was not found above low levels in any tissue (4–15%).

All MGT tissues highly expressed mRNA for Ent1, except for the caput of the epididymis which had moderate mRNA expression (79%). Ent2 mRNA levels were moderate for all MGT tissues ranging from 24 to 82% of control (Fig. 2). Cnt1 was found to only be slightly expressed in any of the MGT tissues (0.5–10%), but Cnt2 was found at mild levels throughout (28–56%). The epididymis caput had more expression of Cnt3 compared to kidney (228%), while the cauda expression levels were higher than that of the kidney (127%).

Interestingly, Mrp1 was found to be highly expressed in the epididymis, particularly in the caput (143%), however all tissues expressed Mrp1 at levels ranging from 57 to 78% (Fig. 3). Conversely, the epididymis had almost no detectable expression of either Mrp2 or and Mrp3. The seminal vesicles had the highest expression of all the MGT tissues for both Mrp2 (56%) and Mrp3 (79%) and the vas deferens demonstrated 42% and 74% expression compared to control for Mrp2 and Mrp3, respectively. The caput and cauda showed marked differences in expression of Mrp4 with the cauda

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