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Research paper

Quantitative PCR analysis and protein distribution of drug transporter genes in the rat cochlea

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ABSTRACT

Membrane transporters can be major determinants in the targeting and effectiveness of pharmaceutical agents. A large number of biologically important membrane transporters have been identified and localized to a variety of tissues, organs and cell types. However, little is known about the expression of key membrane transporters in the inner ear, a promising site for targeted therapeutics, as well as a region vulnerable to adverse drug reactions and environmental factors. In this study, we examined the levels of endogenous membrane transporters in rat cochlea by targeted PCR array analysis of 84 transporter genes, followed by validation and localization in tissues by immunohistochemistry. Our studies indicate that several members of the SLC, VDAC and ABC membrane transporter families show high levels of expression, both at the RNA and protein levels in the rat cochlea. Identification and characterization of these membrane transporters in the inner ear have clinical implications for both therapeutic and cytotoxic mechanisms that may aid in the preservation of auditory function.

1. Introduction

The therapeutic effectiveness of many drugs and biologically active agents is critically dependent on their access to and uptake into specific tissues within the body. The cellular uptake of most drugs is a highly selective process carried out in large part by a diverse array of membrane-bound transporters which generate cellular levels that are therapeutically effective or in some cases toxic. It has been estimated there are over 1500 human transporter genes which encode transporters or transport-related proteins that regulate the cellular uptake, sequestration and efflux of numerous physiological active endogenous and foreign compounds (Drake et al., 2014). Defective transporters can provoke a variety of pathological conditions (Camacho et al., 2003; Kobayashi et al., 2012; Seow et al., 2004) because of their deleterious influence on cellular levels of endogenous substrates as well as by altering the uptake and elimination of drugs (Letschert et al., 2004; Roth et al., 2012; Thompson et al., 2011; Torres et al., 2011). The functional performance of these transporters is genetically controlled and genetic polymorphisms can result in reduced expression or altered performance (De Mattia et al., 2013; Drake et al., 2014; Ishikawa et al., 2005). The extent to which these transport proteins contribute to the accumulation of drugs and other foreign agents is often influenced indirectly by a variety of aberrant biochemical and molecular processes which adapt to cellular demands placed on it by the nutritional requirements of endogenous substances. The expression of these transporters can similarly be modified by drugs, sometimes with deleterious consequences.

The solute carrier (SLC) superfamily of transporters represents one example of essential membrane-bound proteins responsible for trafficking a diverse set of endogenous and exogenous molecules and drugs. The SLC transporters are responsible for the translocation of selective substrates across biological membranes through a variety of biochemical processes that include facilitated diffusion, ion coupling, and ion exchange which participate in the delivery of drugs. In some cases, this process is triggered by an ion gradient that is maintained by active transporters of the ATP-binding cassette (ABC) superfamily of transporters (Greenberg, 2013; Hawley et al., 2013; Wen et al., 2013). Thus, both the SLC and ABC family of transporters represent important proteins

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required for the selective delivery, distribution and behavior of many molecules and therapeutic drugs within different organs of the body.

One sensory organ that has received relatively little attention regarding transporters and drug accumulation is the inner ear, even though numerous studies indicate that a variety of therapeutic agents can affect hearing. Surprisingly, the few studies that have assessed the distribution and levels of drug transporters within different portions of the inner ear suggest that uptake is a highly selective process. For example, recent studies in our laboratory have shown that the relative levels and distribution of three divalent cation SLC transporters, SLC11A2 (divalent metal transporter 1), SLC39A8 (ZIP8) and SLC39A14 (ZIP14) vary within the stria vascularis, organ of Corti and spiral ganglion of the cochlea. Individual variations in the levels and/or localization of these and other transporters involved in delivery and export of drugs in the cochlea as well as the presence of functionally inactive mutant forms of these proteins likely account for the degree to which drug-induced hearing impairment is observed. Therefore, it is important to examine the endogenous levels of the different cellular transport systems within the three cellular compartment of the cochlea in order to understand their potential role in regulating pharmacological activity and ototoxicity. To accomplish this, quantitative real-time quantitative PCR (qRT-PCR) studies were performed to analyze the expression of a focused panel of 84 transporter genes in the stria vascularis, basilar membrane and spiral ganglion neurons of the cochlea.

2. Materials and methods

2.1. Animals

Eight male SASCO Sprague-Dawley rats with average weight of 350 g from Charles River Laboratories (Wilmington, MA) were used for this study. The experimental protocol was reviewed and approved by University at Buffalo Institutional Animal Care and Use Committee.

2.2. Total RNA isolation and cDNA construction

The photomicrograph in Fig. 1A *shows a cross section of the cochlea; the colored lines show the approximate boundaries of the stria vascularis (SV, red line) tissue sample, basilar membrane tissue sample (BM, green line) and modiolus (MOD, yellow) tissue sample used for the gene array studies. All samples were consistently and carefully dissected out in an RNAase free environment.* Total RNA was isolated using RNeasy extraction kit (Qiagen) following the manufacturers protocol. Tissue samples were homogenized in RNA free tubes containing QIAzol lysis reagent and centrifuged at 12,000 g at 4 °C for 15 min. The aqueous phases of the samples were transferred to new tubes and an equal volume of 70% ethanol was added and mixed with the samples and transferred to the RNeasy column. The column was incubated with DNase I for 15 min at room temperature to avoid genomic DNA contamination and afterwards total RNA was eluted using 30 μl RNase-free water. Total RNA concentration and purity were measured using a spectrophotometer (Beckman Coulter DU 640 or Thermo Scientific, NanoDrop 2000). Equal concentrations of total RNA (50 ng) were used to construct cDNA using a RT^2 first strand cDNA kit (Cat #: C-03, SA Bioscience Corporation). SuperArray RT qPCR Master Mix (SA Bioscience Corporation) was used for the PCR reaction.

2.3. Gene expression

The drug transporter RT^2 Profiler™ PCR Array (PARN-070Z-SABio-sciences Corp., MD, USA) was used to investigate the expression pattern of genes involved in absorption, distribution, metabolism and excretion of drugs. Since this gene array contains a focused panel of genes related to drug transport, we used this array to investigate the distribution of gene expression in three different cochlear regions. The RT^2 Profiler™ PCR array contained 84 drug transporter genes including genes that regulate the ATP-binding cassette membrane transportation, solute carrier group of membrane transportation, and other types of ionic and voltage gated transportation. In addition, the array has primers for 5 housekeeping genes (ACTB, RPL13A, HPRT1, LDHA, and RPLP1) to facilitate normalization, a genomic DNA primer to detect genomic DNA contamination, 3 reverse transcription controls and 3 positive PCR controls to test the efficiency of cDNA conversion as well as PCR reaction. The PCR reaction was carried out using SYBR Green fluorescence (SABiosciences) technology with 25 μl per reaction in Bio-Rad MyiQ Single Color Real Time PCR System. The PCR program consisted of one cycle of a hot start (95 °C) for 10 min to activate the DNA polymerase and 40 cycles of amplification (95 °C for 15 s, 60 °C for 1 min). Cycle threshold (C_T) values were measured for each gene on the array. *Three repeats of pooled samples were performed for each condition.*

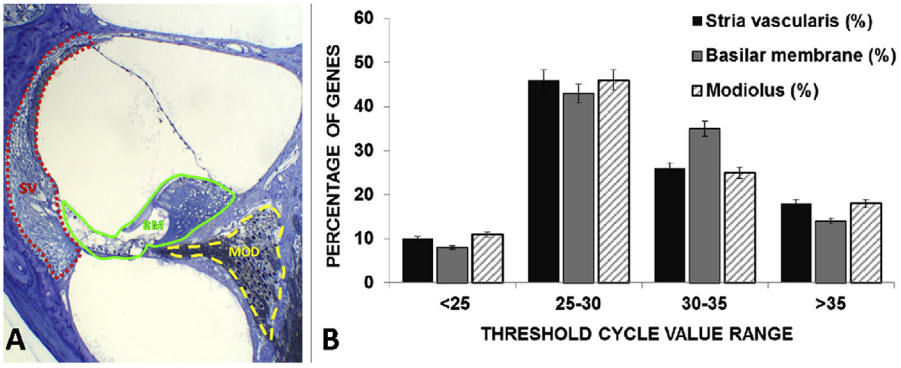

Fig. 1. (A) Photomicrograph of radial section of the cochlea with colored lines demarcating the approximate boundaries of the stria vascularis (SV, red), basilar membrane (BM, green) and modiolus (MOD, yellow) tissue compartments used for qRT-PCR analysis. (B) Percentages of the 84 drug transporter genes with qRT-PCR thresholds cycle (Ct) values in the ranges shown on abscissa. Of the 84 drug transporter genes approximately 85% were classified as detected (Ct $\leq$ 35); ~10% were expressed at high levels (Ct < 25), ~45% at moderate levels (Ct 25–30) and ~28% at low levels (Ct 30–35). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)