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Organic anion transport pathways in antiviral handling in choroid plexus in Oat1 (Slc22a6) and Oat3 (Slc22a8) deficient tissue

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HIGHLIGHTS

- Transport of drugs by the choroid plexus (CP) is believed to be an important factor in regulating CNS drug levels.
- The organic anion transporters Oat1 (SLC22A6) and Oat3 (SLC22A8), as well as SLC22A17, are highly expressed in CP.
- Ex vivo transport of antivirals used for HIV and other diseases was studied in CP from Oat1 and Oat3 knockouts.
- Zidovudine, acyclovir, tenofovir and lamivudine were shown to interact with both Oat1 and Oat3.
- Specific inhibitors of Oat1 and Oat3 may be helpful in altering CNS drug levels by blocking Oat function in the CP.

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ABSTRACT

Transporters in the choroid plexus (CP) regulate transport of numerous compounds of physiological and therapeutic interest between blood and CSF and thus likely play a key role in determining CNS levels of drugs, toxins and metabolites. Here, high CP expression was noted for the organic anion transporters, Oat1 (SLC22A6 or NKT) and Oat3 (SLC22A8) which are also the principal Oats in the renal proximal tubule, as well as SLC22A17, hypothesized to be involved in iron transport. Because Oat1 and Oat3 have overlapping substrate specificity, ex vivo preparations of CP from Oat1$^{(-/-)}$ and Oat3$^{(-/-)}$ mice were used to isolate the individual transport function of each, respectively. Tissue from either knockout mouse mediated the probenecid-inhibitable transport of the Oat substrate, 6-carboxyfluorescein (6CF), confirming the presence of Oat1 and Oat3 function. Because many antiviral medications are Oat substrates, including those crucial in the treatment of HIV infections, the interaction of the antivirals zidovudine, acyclovir, tenofovir, lamivudine, and stavudine, with Oat1 and Oat3 in CP, was investigated by determining the inhibition of 6CF uptake. All the antivirals tested manifested significant interaction with both Oat1 and Oat3, with the exception of stavudine which did not significantly affect Oat1 function. These results could have important implications for antiretroviral (and other drugs) penetration into or retention within the CNS, a major reservoir for virus during HIV infection. Apart from any effect at the blood brain barrier (BBB), designing specific inhibitors of Oat1 and Oat3 may be helpful in altering CNS drug levels by blocking organic anion transporters in the CP. The role of SLC22A17 in the CP deserves further exploration. The ability of Oats to regulate the movement of small molecules across the BBB, CP, proximal tubule and other tissues may also be important for their role in remote sensing and signaling [1,21]).

1. Introduction

Localization of organic anion and cation transporters in the brain and choroid plexus (CP), which functions in the removal of toxins, drugs and metabolites from the cerebrospinal fluid (CSF), has been previously described [10,17]. The organic anion transporters (Oats) located in the CP handle a wide variety of organic anions, including *para*-aminohippurate (PAH), estrone sulfate, benzylpenicillin [9], in a mechanism similar to that present in the kidney, although it is functionally reversed [14]: Oat1/Slc22a6 and Oat3/SLC22a8 which

Abbreviations: Oat1, organic anion transporter 1/NKT/Slc22a6; Oat3, organic anion transporter 3/Slc22a8; CP, choroid plexus; CSF, cerebrospinal fluid; BBB, blood brain barrier.

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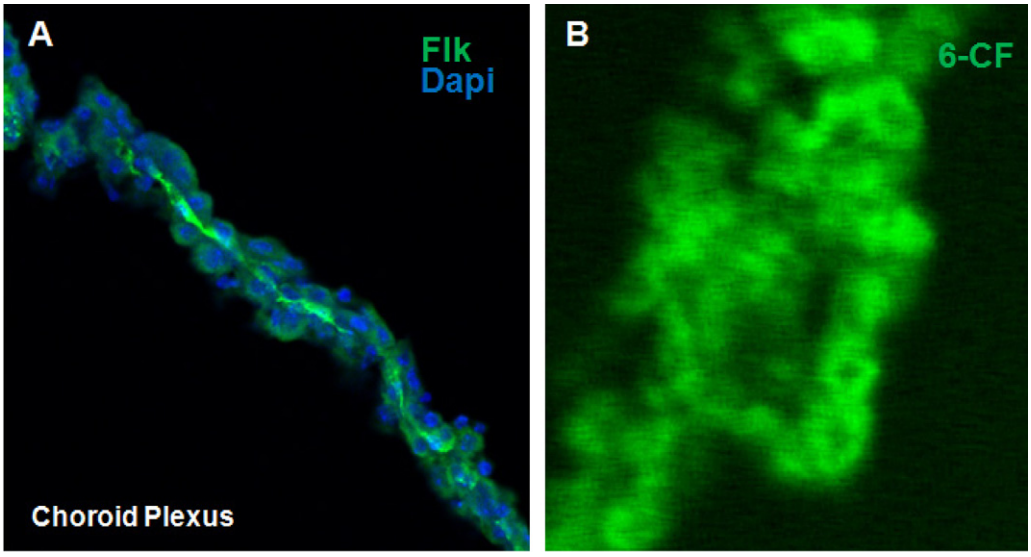

Fig. 1. (A) Confocal fluorescent photomicrograph of isolated adult mouse CP indicating presence of Flk1 (green) in the endothelium of the CP. Dapi (blue) staining indicating nuclei of surrounding CP epithelial cells. (B) Fluorescent photomicrograph demonstrating the uptake of 6CF into isolated adult wild-type mouse CP. (For interpretation of the references to color in text, the reader is referred to the web version of the article.)

are found on the basolateral surface of the kidney proximal tubule [10], function in clearing endogenous metabolites, toxins and drugs from blood to the urine utilizing a tertiary active transport mechanism and a similar mechanism mediates movement of organic anions across the *apical* membrane of the CP [18]. This secretory epithelial tissue is found in the ventricles of the brain where it is believed to regulate the flow of drugs and toxins in and out of brain tissue, the CSF, and central nervous system (CNS). While transport in the CP has remained relatively unexplored due to its smaller size and comparative inaccessibility [14], the generation of knockouts for Oat1 and Oat3 has provided in vivo evidence for Oat-mediated drug transport in this tissue [17,18].

Antiretroviral drug availability in all areas of the CNS is essential for the efficacy of treatment in HIV patients [3]. Drug distribution and effects are directly related to antiretroviral uptake into and removal from the CNS at the CP. Previously, we demonstrated the interaction of HIV antiretrovirals with Oat1 and Oat3 [19]. A similar transport mechanism of nucleoside analogs has also been described in the CP [15]. In the present study, we have investigated the interaction of various antiretrovirals with the organic anion uptake system using a known tracer for these Oats, 6-carboxyfluorescein (6CF), in CP derived from mice deficient for either Oat1/Slc22a6 or Oat3/slc22a8.

2. Materials and methods

2.1. General

Water-soluble probenecid was purchased from Molecular Probes (Carlsbad, CA); 6-carboxyfluoroscein was obtained from Sigma–Aldrich (St. Louis, MO). Stavudine (d4T), lamivudine (3TC), tenofovir, zidovudine (AZT) and acyclovir were purchased from either Moravek Biochemicals (Brea, CA) or Sigma–Aldrich. Flk antibody was purchased from Sigma–Aldrich. Knockout (KO) and wild type (WT) mice were generated as previously described [6]. Oat1 KO mice were back-crossed for 7 generations to C57BL/6J; Oat3 KO mice were back-crossed for 4 generations to C57BL/6J, and C57BL/6J mice were used as wild-type (WT) controls. All experiments were performed on age matched adult female (Oat1 and Oat3 KO) mice between 12 and 20 weeks of age.

2.2. Adult CP uptake assay, imaging, and PCR

Isolation of the choroid plexus was performed as previously described [17,18]. For uptake assays, each CP was cut into 2 pieces and incubated for 1 h at RT in 1 μM 6CF with or without probenecid (2 mM), stavudine (2 mM), acyclovir (2 mM), tenofovir (2 mM), zidovudine (2 mM) or lamivudine (2 mM), respectively. Tissues were then washed with ice cold PBS 4–5 times, frozen in OCT compound and cryosectioned. The 30 μM sections were immediately mounted on slides and imaged with a Nikon confocal microscope. For PCR, CP was isolated from wild type C57BL6 mice, and PCR products of Slc22 genes were resolved on agarose gel by electrophoresis.

2.3. Image acquisition and analysis

30 min prior to data collection the confocal microscope was turned on and allowed to equilibrate. All imaging parameters including lens, aperture and gains were manually set to predetermined values to generate image intensities that fell in the middle of the dynamic range. Digital images were obtained and signal intensity was than measured by averaging 15 points of intensity in well-focused portions along the choroid plexus sample image using NIH image processing software ImageJ.

2.4. Ethics statement

All work was done in accordance with animal protocols approved by the Institutional Animal Care and Use Committee of the UCSD. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

3. Results

3.1. Isolation of CP

To establish whether the tissue isolated from the brain is indeed CP, immunohistochemical staining was carried out with primary antibody against a receptor for vascular endothelial growth factor A, Flk, followed by probing with a fluorescein conjugated secondary