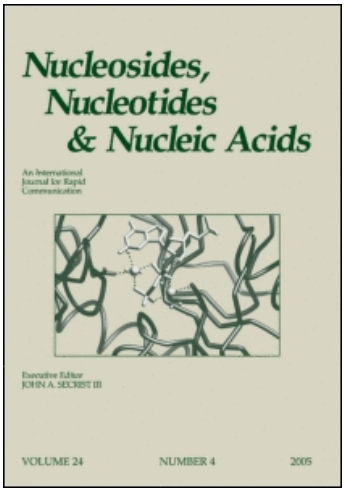

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Renal Deoxyadenosine Transport and Immunodeficiency Associated with Adenosine Deaminase Deficiency

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RENAL DEOXYADENOSINE TRANSPORT AND IMMUNODEFICIENCY ASSOCIATED WITH ADENOSINE DEAMINASE DEFICIENCY

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ABSTRACT

Accumulation of deoxyadenosine (or possibly adenosine) is thought to mediate the immune defect associated with adenosine deaminase deficiency. It is postulated that deoxyadenosine is particularly immunosuppressive in the neonate due to an undeveloped renal secretory mechanism.

Nucleosides and Immunodeficiency Disease. In the absence (or inhibition) of ADA,[1] Ado and dAdo accumulate.[2] These nucleosides and many of their analogs are growth inhibitory or cytotoxic to cells in culture. In the case of genetic deficiency of the enzyme, a syndrome characterized by reduced cellular and humoral immune function results (SCID/ADA$^-$). Although a number of possible mechanisms for the immune-deficiency have been proposed, it is generally agreed that accumulation of dAdo (or perhaps Ado) leads to the immunodeficiency.[3]

The Kidney. The mammalian kidney plays a major role in the maintenance of an extracellular fluid compatible with life.[4] It achieves this function by the selective retention of some substances (i.e., glucose, sodium) and the selective excretion of others. In general, all small molecules (including nucleosides) not bound to plasma proteins are filtered passively by the glomeruli. In addition to being filtered, some substances are actively removed from plasma and secreted across proximal tubular cells into the lumenal fluid (renal secretion) whereas other substances are actively removed from the proximal tubular fluid and transported across the cells into the plasma (renal reabsorption). At

least two major secretory systems have been characterized-the organic cation and organic anion systems. Cimetidine is a potent inhibitor of the organic cation carrier whereas probenecid is a classical inhibitor of the organic anion carrier.

Nucleosides and the Kidney. Mammalian kidneys handle Ado and dAdo differently. After filtration, Ado is reabsorbed, whereas dAdo is secreted.[2] Two major distinctions exist regarding the renal transepithelial transport of these nucleosides and their mediated transport across cell membranes: renal reabsorption and secretion require energy (active transport), and one or the other of the renal processes is highly selective, as Ado differs from dAdo only by the presence of a hydroxyl group in the 2'-position of the sugar moiety. In contrast, the facilitated diffusion (NBTI-sensitive) of these nucleosides across cell membranes occurs via a relatively nonselective carrier.[5]

Purpose. This report summarizes experiments performed to determine the mechanisms for the renal handling of Ado-related compounds. The results suggest that the mechanism for the renal reabsorption of Ado is unique. In the case of dAdo secretion, a model is proposed that is consistent with the demonstrated effects of specific transport inhibitors.

Methods. Renal secretion and reabsorption were studied in humans and mice using a classic clearance technique.[2] In this procedure, the renal clearances of nucleosides were compared with that of creatinine or inulin (a measure of glomerular filtration rate). The role of classic transport systems in the renal handling of nucleosides was studied by administering selective inhibitors to mice prior to the urine collection interval.[6] The inhibitors used were phlorizin (the glucose reabsorption carrier), cimetidine (renal organic cation secretory system), and NBTI and dipyridamole (nucleoside facilitated diffusion). In addition, adenine and 7-deazaadenine derivatives were tested for their abilities to inhibit the active uptake of a classic organic cation carrier substrate, TEA, by mouse kidney slices.[7] Partition coefficients were measured by the distribution at equilibrium of the test compounds between 0.1M phosphate buffer (pH 7.4) and octanol using HPLC with UV detection.

Renal Handling of Ado and dAdo. Initial determinations of renal clearance in a child lacking ADA and in patients treated with DCF (a potent ADA inhibitor) suggested that Ado is reabsorbed whereas dAdo is

secreted, i.e., the clearance values were less or greater than the measured creatinine clearances, respectively.[2] The handling of Ado and dAdo by mouse kidney was confirmed to be qualitatively similar to that in humans. Secretion of dAdo was proved by establishing that the kidney does not significantly synthesize the excreted nucleoside and that "ion-trapping" did not account for the net excess of excreted dAdo over that filtered.[2] dTub was selected as a model compound to study the renal secretion of dAdo because dTub is not a substrate for ADA and is not metabolized significantly by tissues.[7] In mice, dTub undergoes net renal secretion, as does dAdo. It was established unequivocally that dTub is simply a substrate for the organic cation secretory system, as follows: 1) net renal secretion of TEA and dTub were both inhibited by cimetidine; 2) uptake of dTub by mouse kidney slices was inhibited by substrates for the organic cation carrier but not by substrates for the organic anion carrier; and 3) dTub inhibited the active uptake of TEA, but it did not inhibit the uptake of p-aminohippurate (a classic substrate for the organic anion carrier). In mice, neither the renal secretion of dAdo nor the reabsorption of Ado was inhibited by cimetidine or phlorizin.[6] NBTI and dipyridamole did inhibit the secretion of dAdo, but they did not inhibit Ado reabsorption. These data suggest that the renal reabsorption of Ado may be unique, i.e., independent of the known carriers for glucose, organic cations, and nucleosides. In contrast, dAdo secretion appears limited by the nucleoside facilitated diffusion carrier sensitive to NBTI and dipyridamole.

A Model for dAdo Secretion. Like the nucleoside transport carrier in cell membranes, the renal organic cation secretory system inhibited by cimetidine is relatively nonselective in its structural requirements. Since the pKa values for dAdo and dTub are similar,[7] it appears unlikely that dTub would be secreted by this system whereas dAdo is not. A possible explanation for the data to date became apparent when a series of adenine and 7-deazaadenine derivatives were tested as inhibitors of TEA uptake by mouse kidney slices.[6] The ability to inhibit TEA uptake appeared to be associated only with a certain degree of lipid solubility, i.e., dTub but not dAdo had the requisite lipid solubility to produce inhibition (partition coefficient of 0.78 versus 0.27, Table 3 of reference 6). A model to explain these data is presented below.

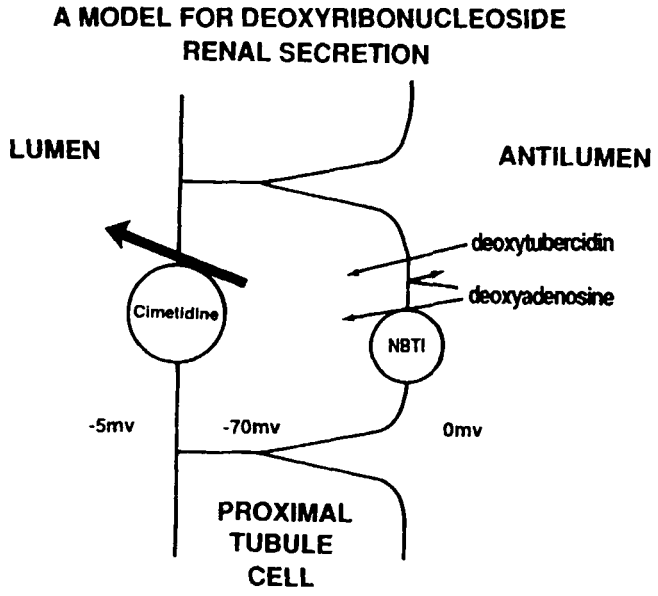

FIGURE 1

According to Kinsella, et al.,[8] the active transport of organic cations should occur at the lumenal membrane. That is, organic cations should enter renal proximal tubular cells via the antilumenal membrane down their electrochemical gradient; however, energy must be expended to transport the cations into the lumenal fluid. Due to its poor lipid solubility, it is proposed that the secretion of dAdo is limited by its rate of entry into the proximal tubular cell by the NBTI-sensitive transporter, rather than by the lumenal transporter sensitive to cimetidine. In contrast, dTub has sufficient lipid solubility such that its secretion is limited by the cimetidine-sensitive carrier.

dAdo Renal Secretion and Immunodeficiency Disease. The model above proposes that dAdo is secreted by the renal organic cation carrier. This system is not developed at birth in mice, dogs or pigs.[9] In fact, several days are required for the development of this transport system to its adult capacity. Assuming that dAdo is secreted by this system and that a similar neonatal development occurs in humans, neonates may be particularly susceptible to the effects of dAdo. Further, renal blood flow in the newborn human is only approximately 5-6% of the cardiac

output, compared to 15-25% in adults,[10] a factor that would also compromise the neonate's ability to clear this nucleoside. If true, it may be possible to protect SCID/ADA$^-$ patients by intervening during the early days of life, allowing time for the development of renal and immune function.

ACKNOWLEDGMENT

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1. The abbreviations used are ADA, adenosine deaminase; Ado, adenosine; DCF, 2′-deoxycoformycin; dAdo, 2′-deoxyadenosine; dTub, 2′-deoxytubercidin; NBTI, p-nitrobenzyl-6-thioinosine; SCID/ADA$^-$, severe combined immunodeficiency disease associated with ADA deficiency; and TEA, tetraethylammonium.

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