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Renal Handling of 2′-Deoxyadenosine and Adenosine in Humans and Mice

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Summary. *In a child lacking adenosine deaminase and in patients treated with deoxycoformycin (a potent inhibitor of the enzyme), apparent renal secretion of 2′-deoxyadenosine (dAdo) and reabsorption of adenosine (Ado) were observed. The renal clearance of dAdo in humans was approximately five-fold that of creatinine, whereas the renal clearance of Ado was only one-fifth that of creatinine. In mice treated with deoxycoformycin, a similar paradigm was observed. Specifically, plasma levels of Ado and dAdo were elevated to detectable levels and apparent renal secretion and reabsorption of these purine nucleosides became manifest. Thus, the mouse may serve as a suitable model to study the renal handling of these two compounds. The active renal secretion of dAdo may occur because the compound has not been appreciably synthesized by mouse kidney in situ, and 'ion-trapping' of dAdo in acid urine could not explain the net secretion. The differential transport of these similar purine nucleosides suggests a very selective transport system in mammalian kidney. Although carrier-mediated, facilitated diffusion of purine nucleosides across cell membranes is a well-known phenomenon, the present data indicate the existence of (an) active transport sysem(s) for the transepithelial secretion of dAdo, and possibly for the reabsorption of Ado.*

Introduction

A large number of purine nucleoside analogs have been synthesized and tested for antitumor and other biological activities [3]. Since there is an exhaustive body of information regarding purine nucleosides, it is surprising that little is known regarding the renal handling of purines other than the oxypurines (uric acid, xanthine, hypoxanthine).

Recently, two purine enzyme deficiencies have been noted to be associated with inherited immunodeficiencies. Absence of ADA is considered to be causally related to severe combined immunodeficiency, a clinical syndrome in which patients are extremely deficient in cellular and humoral immune responses [13]. A second purine enzyme deficiency, PNPase deficiency, is associated with the loss of cellular or T cell-mediated immunity [19]. In both cases, accumulation of endogenous purine nucleoside substrates occurs and it is thought that these compounds may mediate the lymphocyte dysfunction [21].

ADA and PNPase normally serve in the catabolic pathway of purine metabolism (Fig. 1). Since some of the enzyme substrates which accumulate (i.e., Ado and dAdo in ADA deficiency and 2′-deoxyguanosine in PNPase deficiency) are known to produce cellular toxicity in tissue culture, it is reasonable to propose that the kidney may have evolved mechanisms for the elimination of some of these purine nucleosides. Purine nucleosides are transported across cell membranes by a facilitated diffusion mechanism [18]. This system is relatively nonspecific in its structural requirements, and it is not an active, metabolically dependent process. While examining the purine levels in a child with ADA deficiency, we were struck by the large amounts of dAdo which were excreted in his urine [10]. This paper describes in a quantitative fashion the renal handling of dAdo and Ado in humans and mice. The results suggest that active transport systems exist in the mammalian kidney for the secretion of dAdo and for the reabsorption of Ado. Preliminary reports of portions of this work have appeared and been prepared in abstract form [11, 12].

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The abbreviations used in this paper are: ADA, adenosine aminohydrolase EC 3.5.4.4; Ado, adenosine; dAdo, 2′-deoxyadenosine; DCF, 2′-deoxycoformycin; HPLC, high-performance liquid chromatography; PBS, 0.14 *M* NaCl plus 0.01 *M* phosphate buffer, pH 7.4; PNPase, purine nucleoside: orthophosphate ribosyltransferase EC 2.4.2.1; SCID : ADA$^-$, severe combined immunodeficiency disease associated with ADA deficiency

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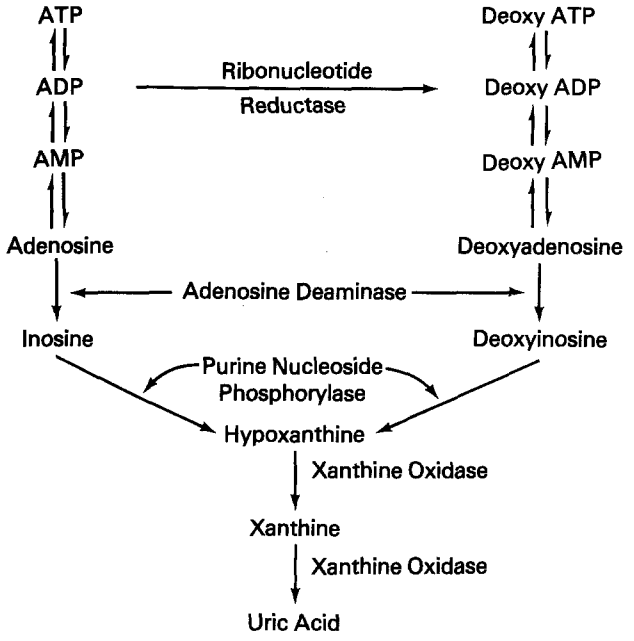

Fig. 1. Pathways of dAdo and Ado metabolism

Materials and Methods

Materials. 2′-Deoxycoformycin was obtained from the Drug Synthesis and Development Branch, National Institutes of Health, Bethesda, MD, USA. 8-[^{14}C]-2′-deoxyadenosine (45 mCi/mmol), 8-[^{14}C]-adenosine (50 mCi/mmol) and 8-[^{3}H]-adenosine (18 Ci/mmol) were purchased from Moravek Biochemicals Corp., City of Industry, CA, USA. 8-[^{3}H]-2′-Deoxyadenosine (11 Ci/mmol) was purchased from ICN Chemical and Radioisotope Division, Irvine, CA, USA. Inulin-[^{14}C]-carboxylic acid (10 mCi/mmol) was a product of Amersham Corporation, Arlington Heights, IL, USA. Purine nucleosides and bases were obtained from P & L Biochemicals, Milwaukee, WI, USA. Methazolamide was obtained from Lederle Laboratories, Pearl River, NY, USA. Chloroacetaldehyde and dimethylacetal were purchased from Aldrich Chemical Co., Milwaukee, WI, USA.

Renal Clearance Determinations. Blood and urine samples were obtained from a male child with SCID : ADA$^-$ treated with extensive erythrocyte transfusion therapy and maintained in reverse isolation [20]. Collections were made during 8-h or 24-h intervals at times when the patient was clinically free of infection. Plasma and urine were also collected from five cancer patients during treatment with DCF, a potent ADA inhibitor [1], in a phase I–II clinical trial [2]. The cancer patients ranged from 25–65 years of age (4 females and 1 male) and all had received extensive prior chemotherapy for their advanced solid tumors. Additional clinical aspects of the phase I–II clinical trial will be presented elsewhere. Plasma and urine samples were collected immediately prior to DCF administration and during a 24-h interval thereafter. In these patients, adenine compounds were measured in neutralized perchloric acid extracts of plasma and urine by HPLC with fluorescense detection as described below. Creatinine was measured colorimetrically as described by Smith [24].

Renal clearance measurements in mice were performed as described by Konikowski et al. [9]. Male AKR mice weighing 25–30 g each received an IP injection of DCF (5 mg/kg). An SC injection of [^{14}C]-inulin (10 mCi/mmol) was administered 10 min later. Each animal was induced to void its bladder by retroperitoneal massage prior to urethral ligation 30 min after DCF administration. Blood was collected from the retro-orbital sinus of each mouse and placed in heparinized vials 60 min after DCF administration. The mice were then sacrificed by cervical dislocation, the bladder was excised immediately and its contents were washed with 10 ml PBS into a 50-ml centrifuge tube. Whole blood and urine of two animals were pooled for each determination. Plasma was obtained by centrifugation at 450 *g* for 10 min at 4° C. The plasma was extracted with 0.4 *N* perchloric acid at 4° C. The acid-soluble fraction obtained after centrifugation was immediately neutralized with 10 *N* KOH and the insoluble potassium perchlorate was removed by centrifugation. dAdo and Ado were measured in the neutralized plasma extracts and urine by means of HPLC as described below. The renal clearances were calculated as follows:

$$\text{Renal clearance (ml/min)} = \frac{\text{Urinary excretion rate (nmol/min)}}{\text{Plasma concentration (nmol/ml)}} .$$

In one experiment in which animals were given 8-[^{3}H]-dAdo, the renal clearance was determined by radioactivity measurements as well as the HPLC method described below. Radioactivity in plasma and urine was measured by liquid scintillation spectrometry with a Beckman Model LSC7500 Instrument (Beckman Instruments, Inc., Irvine, CA, USA). 'Midpoint' values of the plasma inulin concentration and the plasma 8-[^{3}H]-dAdo concentration were estimated from measured values of these compounds at 60 min and historical plasma decay curves, as described by Konikowski et al. [9]. Renal clearances of dAdo, Ado, and inulin were also determined in mice after treatment with methazolamide (25 mg/kg), a carbonic anhydrase inhibitor. Urine pH was determined by means of pHydrion pH paper (Micro Essential Laboratory, Brooklyn, NY, USA) with pH ranges of 3.5–6.0, 6.0–8.0, and 8.0–9.5.

Plasma Protein Binding of dAdo and Ado. Outdated human or freshly obtained mouse plasma was preincubated for 1 h at 4° C in the presence of 5 μ*M* DCF (to completely inhibit ADA activity). 8-[^{14}C]-dAdo and 8-[^{14}C]-Ado were then added to give a final concentration of 1 μ*M* or 5 μ*M* (~ 0.05 μCi/ml). Duplicate samples (0.7–2.0 ml) were then transferred into Centriflo membrane cones, type CF-25 (Amicon Corp., Lexington, MA, USA). The samples were centrifuged at 800 *g* for 60 min at 4° C. Radioactivity was measured in aliquots of both plasma and plasma filtrate by liquid scintillation spectrometry. Binding of dAdo and Ado to the membrane cones was determined in the same manner except that PBS was used instead of plasma. Approximately 10% of the total radioactivity was bound to the membrane cones with either nucleoside. To determine the extent of metabolism, the plasma filtrates were pooled and the radioactivity associated with dAdo or Ado was determined by HPLC.

High-Performance Liquid Chromatography. The 1,N^6-ethenoderivatives of adenine compounds were prepared as described by Kuttesch et al. [10]. The derivatives were measured by fluorescence detection after elution from a μBondapak C_{18} reverse-phase column (4 mm × 30 cm; Waters Associates, Milford, MA, USA). A linear gradient from water to water plus methanol was formed with the aid of the apparatus illustrated in Fig. 2. A gradient mixing chamber with a magnetic stirring bar was filled with water to a volume of 60 ml. The column was washed at a flow rate of 2 ml/min for 10 min prior to injection of the sample via a Rheodyne Model

7120 Injector (Rheodyne, Inc., Berkley, CA, USA). At the time of sample injection, 70% methanol in water was pumped into the mixing chamber at a flow rate of 1 ml/min by a Gilson Minipuls-2 Peristaltic Pump (Gilson Medical Electronics, Middleton, WI, USA). The gradient mixing chamber provided solvent via a four-way slide valve (Model FT-144, Glenco Scientific, Inc., Houston, TX, USA) to a Milton Roy Minipump (Model 396-31, Milton Roy, Inc., Riviera Beach, FL, USA). The flow rate of solvent to the column was 2 ml/min. The 1,N^6-ethenoderivatives of adenine, Ado, and dAdo eluted with retention times of approximately 13, 14, and 15 min, respectively. After 20 min, the four-way slide valve was switched in such a way that 70% methanol was delivered to the Minipump to wash the column between samples (10 min at 2 ml/min). During the column-washing interval, the gradient mixing chamber was washed with water by successively introducing water to the chamber and draining the chamber to a waste reservoir via the four way slide valve. After washing, the gradient mixing chamber was filled with water to a volume of 60 ml to repeat the sample analysis cycle. The 1,N^6-ethenoderivatives were monitored by fluorescence detection with a Model FS-970 L.C. Fluorometer (Schoeffel Instruments Division, Kratos, Inc., Westwood, NJ, USA). The excitation wavelength was 280 nm with excitation filter no. 7-54, and emission was monitored with emission filter KV-389. Separation of the 1,N^6-ethenoderivatives under these conditions was essentially as previously illustrated with a different HPLC instrument [17]. Identities of the 1,N^6-ethenoderivatives of dAdo, Ado, and adenine were confirmed in at least one sample of plasma and urine from each patient as follows: (1) Comparison of retention times to those of authentic compounds; (2) requirement of chloroacetaldehyde to form fluorescent derivatives; (3) disappearance of peaks corresponding to 1,N^6-etheno-2′-deoxyadenosine and 1,N^6-ethenoadenosine after treatment of the samples with ADA prior to derivatization; and (4) conversion of dAdo to adenine by incubation of the sample in 0.4 *N* perchloric acid for 1 h at room temperature prior to derivatization.

In mice, dAdo and Ado were measured in plasma extracts or urine by the fluorometric method described above or an HPLC method essentially as described by Hartwick and Brown [6]. In the latter procedure, eluting compounds were monitored by their UV absorbance at 254 nm (UV III Monitor, Laboratory Data Control, Riviera Beach, FL, USA) and at 280 nm (Spectro-II Monitor, Laboratory Data Control). The identities of dAdo and Ado in these samples were confirmed by comparison of their retention times and 254 : 280 peak area ratios to authentic standards. The linear gradient from 10 m*M* KH_2PO_4, pH 5.5–10 m*M* KH_2PO_4 plus 20% methanol was formed in 30 min at a flow rate of 1 ml/min. A Laboratory Data Control LC 7800 System equipped with a Chromatography Control Module II (CCM) with two Constametric pumps was used. The machine-programmed feature of the CCM was used to integrate and plot the output of both UV detectors simultaneously. When samples containing 8-[^{3}H]-dAdo were used, fractions of the eluting material were collected at 2-min intervals into scintillation vials by means of an LKB Model 2112 Fraction Collector (LKB Instruments, Inc., Rockville, MD).

ADA was determined in lysates of mouse red blood cells by measurement of dAdo disappearance and product (deoxyinosine plus hypoxanthine) formation. A reaction mixture containing 200 μ*M* dAdo, 50 m*M* phosphate buffer, pH 7.5, and 0.1% lysate was incubated at 37° C for 30 min. The reaction was terminated by heating the mixture in a boiling water bath for 3 min. The samples were centrifuged and the clear supernatants were analyzed by means of the μBondapak-C_{18} column eluted isocratically with 10 m*M* KH_2PO_4, pH 5.5, plus 10% methanol at a flow rate of 2 ml/min. ADA activity was determined by measurement of both 2′-deoxyinosine (the deamination product of dAdo) and hypoxanthine (the phosphorolysis product of 2′-deoxyinosine).

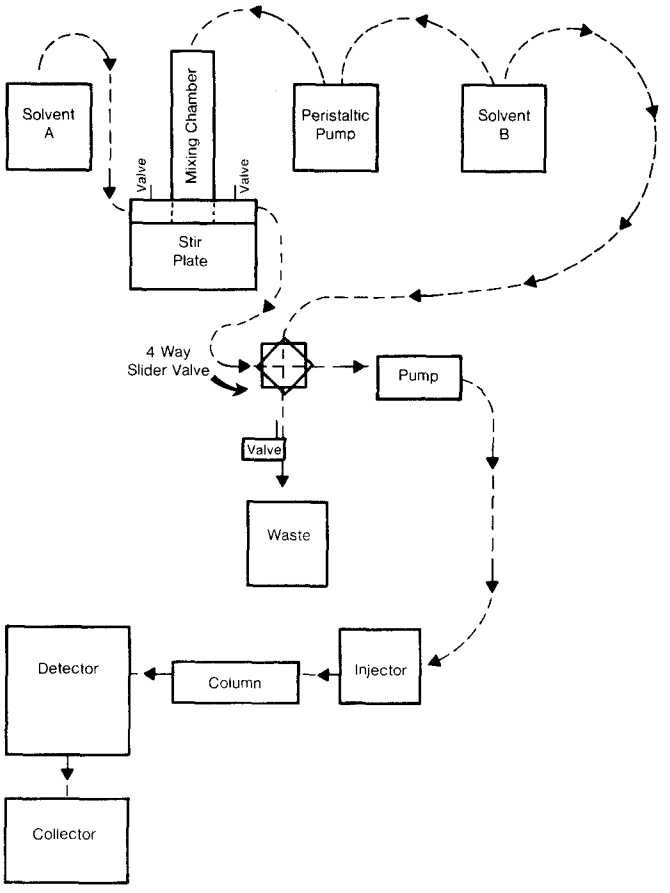

Fig. 2. Block diagram of apparatus used to determine adenine compounds by gradient elution of a reverse-phase column and fluorescence detection (see *Methods*). Solvent A, water; solvent B, 70% methanol

Results

In a SCID : ADA$^-$ patient studied in our laboratory, the urinary excretion of Ado, dAdo, and creatinine was measured at one interval prior (4–7 months) to extensive erythrocyte transfusion therapy and at two intervals during transfusion therapy (29 and 41 months) during the child's life (Table 1). The renal clearance of dAdo was greater than that of Ado at all intervals, which suggested that the renal handling of these similar compounds was different in this patient. The renal excretion of creatinine in the SCID : ADA$^-$ patient was within normal limits [8] for children of the same ages at all three time intervals. At one observation period, the plasma level of creatinine was determined (age 41 months in Table 1). At this time, the renal clearance of dAdo was four-fold greater than the measured creatinine clearance. In contrast, the renal clearance of Ado was less than that of creatinine. The endogenous pools of

Table 1. Renal clearances of dAdo, Ado, and creatinine (Cr) in a child with severe combined immunodeficiency disease and adenosine deaminase deficiency (SCID : ADA$^-$)

Age (months)	Body weight (kg)	Body surface area (m^2)	Plasma concentration (nmol/ml)			Urine concentration[a] (nmol/ml)				Renal clearance (ml/min)			Renal clearance (ml/min/1.73 m^2)		
			Cr	dAdo	Ado	(n)	Cr	dAdo	Ado	Cr	dAdo	Ado	Cr	dAdo	Ado
4–7	4.64	0.28	n.d.	< 0.10	2.20	(7)	1866 ± 460	76.9 ± 28.8	6.7 ± 2.4	n.d.	> 80.0	0.10	n.d.	> 494	0.60
29	6.99	0.37	n.d.	< 0.10	0.40	(4)	2020 ± 450	28.8 ± 7.2	4.2 ± 1.1	n.d.	> 66.5	2.40	n.d.	> 311	11.30
41	8.04	0.41	74.4	< 0.10	0.40	(2)	1315 ± 415	7.1 ± 0.0	2.4 ± 1.1	14.7	> 58.9	5.00	61.9	> 248	21.00

The plasma and urine levels of dAdo, Ado and Cr were measured in a child with SCID : ADA$^-$ during one interval prior to erythrocyte transfusion therapy (4–7 months of age) and two intervals during transfusion therapy (29 and 41 months of age) as described by Kuttesch et al. [10] or Mills et al. [15]. Erythrocyte transfusion therapy in SCID : ADA$^-$ is reviewed by Schmalstieg et al. [20]

[a] The urine flow rates for these intervals were 0.10, 0.23 and 0.83 ml/min (4–7, 29, and 41 months of age, respectively). n = number of 8- or 24-h urine collections

NOTE, n = urine samples

purines in this child have been measured on numerous occasions in several different laboratories [10, 14, 15, 20, 22]. Table 1 presents data obtained during defined urine collection intervals, and the results are representative of the plasma and urine values obtained at other times in this child.

The renal clearances of Ado, dAdo and creatinine were also determined in five cancer patients who had received DCF (Table 2). The plasma concentrations of these substances were relatively constant and elevated during the intervals in which these measurements were made. The renal clearance of dAdo exceeded (approximately two- to ten-fold) that of creatinine in all five patients, whereas the renal clearance of Ado was less than that of creatinine in these patients (Table 2). The binding of 8-[^{14}C]-dAdo and 8-[^{14}C]-Ado to human plasma proteins was less than 5% (Table 3). This indicates that these compounds are probably freely filterable from human plasma when the plasma levels are near 1 μM. A good estimate of the renal clearance of adenine could not be made in all of the patients because adenine levels were not consistently detectable in plasma and urine (Table 2). However, the low levels of adenine indicate that there was insignificant degradation of dAdo to adenine during the extraction and derivatization procedure [22]. The creatinine clearance values are relatively low in the patients studied, a finding which probably relates to their poor state of health. Prior to treatment with DCF, the level of each adenine compound was at or below the limits of detection (indicated by < in Table 2). Other clinical and biochemical findings in the phase I study of DCF will be summarized elsewhere.

Separation of the 1,N^6-ethenoderivatives of adenine, Ado, and dAdo in the urine of patient 5 from Table 2 is illustrated in Fig. 3. The derivatives eluted with retention times of 13, 14, and 15 min, respectively (chromatogram A in Fig. 3). These adenine compounds were not detectable under the described assay conditions in a sample of urine obtained immediately prior to DCF administration (chromatogram B in Fig. 3). Also, chloroacetaldehyde derivatization was necessary to obtain fluorescent peaks at the relevant retention times (chromatogram C in Fig. 3). Treatment of the diluted urine sample with ADA prior to chloroacetaldehyde derivatization eliminated the fluorescent peaks corresponding to Ado and dAdo (chromatogram D in Fig. 3). Incubation of the sample in 0.4 N perchloric acid shifted the fluorescent peak associated with dAdo (15 min) to that associated with adenine (13 min). This result was obtained whether the acid treatment was performed before or after chloroacetaldehyde derivatization, i.e., the acid lability of dAdo and 1,N^6-etheno-2′-deoxyadenosine is confirmed. Under the assay conditions shown, there is about two-fold greater fluorescence of the adenine derivative than of dAdo. Although actual patient urine samples are shown (chromatograms A through E), similar results were obtained with authentic adenine, Ado, and dAdo. Chromatogram F in Fig. 3 illustrates the separation of the authentic 1,N^6-etheno derivatives of the purines of interest.

The above results in humans suggest that there is net secretion of dAdo and net reabsorption of Ado by the human kidney. To pursue this possibility further and to study the mechanisms involved, we have simulated the experiments with mice. The plasma concentrations of dAdo and Ado are elevated following DCF administration to mice (Fig. 4). Although there is only an approximately two-fold

Table 2. Renal clearances of dAdo, Ado, adenine (Ade), and creatinine (Cr) in patients treated with DCF

Patient	DCF dosage[a]	Body surface area (m^2)	(n)	Plasma concentration (nmol/ml)			Urine concentration (nmol/ml)				Urine flow (ml/min)	Renal clearance (ml/min)			
				Cr	dAdo	Ado	Ade	Cr	dAdo	Ado	Ade		Cr	dAdo	Ado	Ade
1	22 mg/24 h	1.42	(2)	59.1 ± 1.2	0.42 ± 1.2	0.20	0.49 ± 0.29	1,733	131	< 3.5	< 2.0	1.14	33.4	355	–	< 4
2	26 mg/24 h	1.74	(4)	93.2 ± 6.7	1.04 ± 0.60	0.62 ± 0.24	0.03 ± 0.01	3,148	148	< 3.5	< 2.0	1.99	67.2	283	< 11.6	< 133
3	2 mg/25 min	2.19	(6)	81.5 ± 14	< 0.07	4.40 ± 2.6	< 0.03	7,351	12	3.5	< 2.0	1.09	98.3	> 187	0.9	–
	30 mg/24 h		(9)	87.8 ± 6.2	0.56 ± 0.32	4.50 ± 2.5	< 0.03	13,360	422	15.6	< 2.0	0.51	77.6	384	1.8	–
4	2.8 mg/15 min	1.42	(5)	67.1 ± 8.1	0.49 ± 0.26	2.30 ± 1.5	0.18 ± 0.15	6,010	118	< 3.5	< 2.0	0.40	35.8	97	< 0.6	< 4.4
5	2.6 mg/30 min	1.56	(4)	45.9 ± 4.4	< 0.07	1.00 ± 0.24	< 0.03	7,764	113	21.5	8.7	0.19	32.1	> 307	4.1	> 55.1

Plasma and urine samples were obtained during 24-h intervals after DCF administration. dAdo, Ado, Ade, and Cr were measured as described in Methods.
Plasma values are given as median value ± range. (n) = number of plasma samples obtained during the 24-h interval
[a] Total amount of DCF administered/infusion interval

Table 3. Plasma binding of 8-[^{14}C]-dAdo and 8-[^{14}C]-Ado in humans and mice

	% Free	% Remaining as dAdo or Ado
Human		
dAdo, 1 μ*M*	96 ± 5	99
Ado, 1 μ*M*	96 ± 4	95
Mouse		
dAdo, 1 μ*M*	97 ± 3	100
Ado, 5 μ*M*	83 ± 4	100

Pooled, outdated human plasma and pooled mouse plasma were preincubated with DCF (5 μ*M*) for 1 h at 4° C prior to addition of the radiolabeled nucleosides at the concentrations shown. Plasma binding was determined by ultrafiltration using Centriflo cones. The values shown are the mean ± SE of three determinations in each-case. The pooled plasma filtrates were analyzed to determine the extent of metabolism of the radiolabeled nucleoside with HPLC

elevation of Ado during a 90-min interval after DCF, the level of dAdo increases from a value less than 0.1 μ*M* to greater than 1.0 μ*M*. ADA activity in mouse red blood cells decreased to less than 5% of control values within 10 min after DCF administration and remained at this low level throughout the 90-min observation period (data not shown). As indicated at the top of Fig. 2, a protocol could be devised whereby the renal clearance of dAdo, Ado, and inulin could be measured during times in which the plasma levels were relatively constant or predictable, i.e., 30–60 min after DCF administration. The renal clearance of dAdo during this interval was greater than the simultaneously measured inulin clearance, whereas the Ado clearance was less than that of inulin (Table 4). Binding of dAdo to mouse plasma proteins was less than 5%; however, a small but significant fraction (17%) of Ado was bound to mouse plasma macromolecules (Table 3). The clearance calculation for Ado in mice, therefore, was corrected to account for this binding (Table 4). It should be noted that this correction increases the absolute clearance value for Ado rather than decreasing it.

To determine whether or not the apparent secretion of dAdo by mouse kidney was a result of synthesis of this compound by the kidney, the experiment summarized in Table 5 was performed. Radioactive 8-[^{3}H]-dAdo was administered to mice and urine clearance determinations were performed in a manner similar to that shown in Fig. 4 and given in Table 4. The dAdo clearance was calculated from measurements of radioactive 8-[^{3}H]-dAdo in plasma and urine as well as measurements of the total pool (HPLC) of dAdo in plasma and urine (Table 5). The

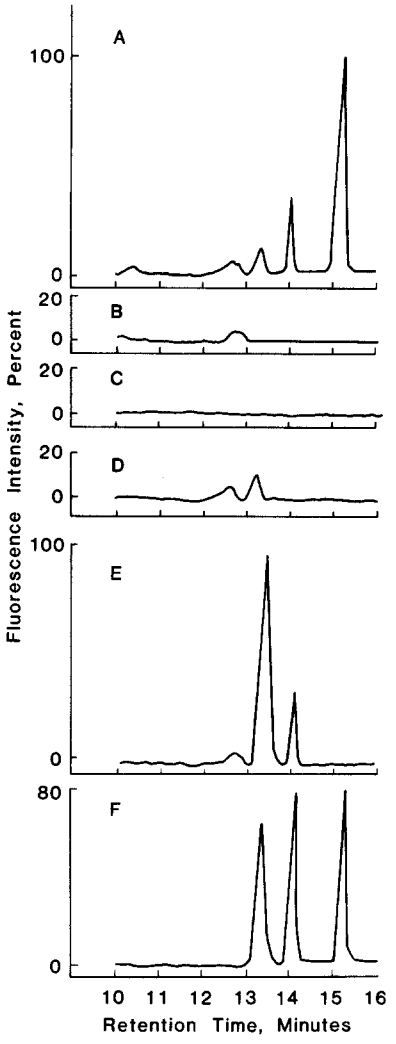

Fig. 3. A–F Verification of Ado and dAdo in the urine of a patient treated with DCF (patient 5 in Table 2). In all cases, 10 µl of a 50-fold diluted aliquot of urine was injected onto the HPLC apparatus shown in Fig. 2. **A** 1,N^6-ethenoderivatives of adenine (13 min), Ado (14 min), and dAdo (15 min) in urine after treatment of the patient with DCF; **B** Urine sample immediately prior to DCF administration; **C** Urine sample after DCF treatment but not derivatized with chloroacetaldehyde; **D** Same urine sample derivatized with chloroacetaldehyde after incubation with ADA; **E** The urine sample incubated in 0.4 *N* perchloric acid for 1 h at room temperature prior to derivatization with chloroacetaldehyde; **F** Authentic 1,N^6-ethenoderivatives of adenine, Ado, and dAdo

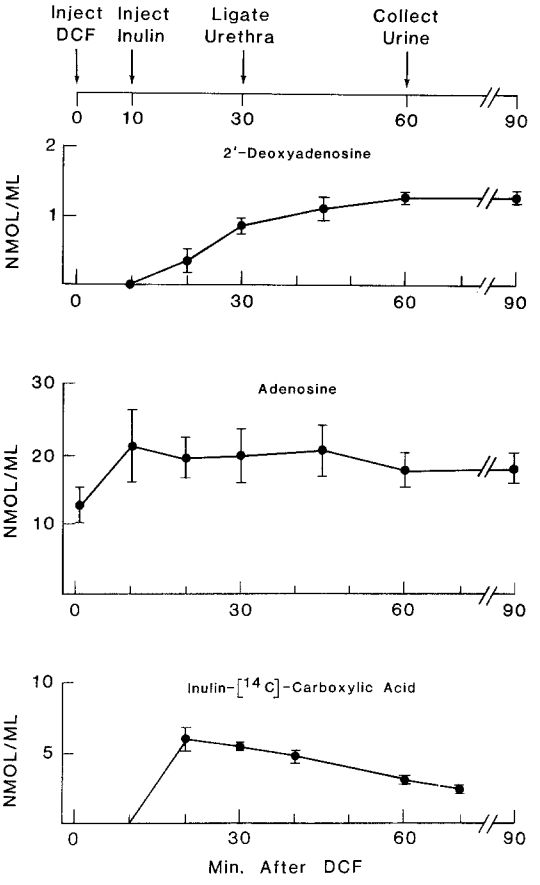

Fig. 4. Plasma concentrations of dAdo, Ado, and inulin [^{14}C]-carboxylic acid in mice after DCF treatment. Male AKR mice were given a single IP injection of DCF (5 mg/kg). At various times after DCF administration, blood was collected from the retro-orbital sinus into heparinized vials. Plasma was obtained by centrifugation. dAdo and Ado were assayed in neutralized, acid-soluble extracts of plasma by HPLC, as described in *Methods*. An SC injection of radioactive inulin (0.05 µCi/g) was administered to the mice 10 min after DCF treatment. The plasma inulin levels were determined by liquid scintillation spectrometry. Each *point* represents the mean ± SE of 4–19 determinations. The interval between urethral ligation (30 min after DCF) and urine collection (60 min after DCF) was used for the renal clearance calculations

renal clearance estimates in both cases were identical. Furthermore, the specific activity of dAdo is identical in plasma and urine, i.e., there is no dilution of the label in urine (Table 5). This suggests that the excess of dAdo observed in urine over the filtered fraction does not result from the synthesis of the compound de novo or from nonradioactive precursors by the kidney. Approximately 30% of the total radioactivity in plasma was [^{3}H]-dAdo, whereas [^{3}H]-dAdo accounted for greater than 90% of the total radioactivity observed in urine in these experiments (data not shown).

Since dAdo is a weak base (pK_a = 3.8) [5], the apparent renal secretion of this compound may result from 'ion-trapping' in an acidic urine. To test this possibility, the renal clearance of dAdo as well as the

Table 4. Renal clearance of dAdo, Ado, and inulin-[^{14}C]-carboxylic acid in mice after DCF treatment

	Plasma concentration (nmol/ml)	Renal excretion (nmol/min)	Renal clearance (ml/min)	Clearance ratio (Cl_x/Cl_{inulin})
2′-Deoxyadenosine or dAdo	1.11 ± 0.10	0.342 ± 0.074	0.313 ± 0.072[b]	1.71 ± 0.17
Adenosine or Ado	14.00 ± 2.58	0.542 ± 0.051	0.059 ± 0.009[a, c]	0.42 ± 0.11
Inulin-[^{14}C]-carboxylic acid	4.33 ± 0.13[d]	0.816 ± 0.140	0.193 ± 0.034	1.00 ± 0.00

Male AKR mice were given DCF (5 mg/kg) and inulin-(^{14}C)-carboxylic acid (0.05 μCi/g) as described in Fig. 4. The urethra of each animal was ligated 30 min after DCF treatment. Blood was collected from the retro-orbital sinus and the animal was sacrificed to obtain the bladder 60 min after DCF treatment. The bladder was washed with 10 ml of phosphate-buffered saline to obtain the urine. dAdo and Ado in neutralized, acid-soluble extracts of plasma and in urine were assayed by HPLC (see Methods). The plasma and urine contents of inulin-[^{14}C]-carboxylic acid were determined by liquid scintillation spectrometry. The results shown are the mean ± SE of 10 determinations in each case

[a] The renal clearance of adenosine was corrected for the plasma binding (17%) of this nucleoside in mice (see Table 3). Other values are not corrected for plasma binding

[b] Significantly greater than the inulin clearance, $P < 0.025$, Student's paired t-test

[c] Significantly less than the inulin clearance, $P < 0.005$, Student's paired t-test

[d] Estimated 'midpoint' value for plasma inulin concentration was determined from the measurement at 60 min after DCF administration and 'historical' data given in Fig. 4. Plasma values for dAdo and Ado were relatively constant during the urine collection interval (Fig. 4) and are not corrected

Table 5. Renal clearance of dAdo in mice

	Method of measurement	Clearance ratios (Cl_x/Cl_{inulin})	Specific activity (DPM/nmol)	
			Plasma	Urine
dAdo	UV absorbance[a]	2.20 ± 0.04	–	–
8-[^{3}H]-dAdo	Liquid scintillation counting[b]	2.29 ± 0.12	5,604 ± 281	5,847 ± 246

Male AKR mice were treated as described in Table 4. The mice received an SC injection of 8-[^{3}H]-dAdo (100 nmol/g; specific activity, 2.5 nCi/nmol) 10 min after DCF administration. Renal clearances were calculated from data obtained by chemical measurements of dAdo and by radioactivity measurements of 8-[^{3}H]-dAdo. The values shown are the mean ± SE of three determinations in each case

[a] UV absorbance of HPLC analysis of plasma and urine measured at 254 nm

[b] Radioactivity of HPLC fractions corresponding to dAdo in plasma and urine

Table 6. Effect of urine alkalinization on the renal clearance of dAdo, Ado, and inulin-[^{14}C]-carboxylic acid in mice

	Plasma concentration (nmol/ml)	Renal excretion (nmol/min)	Renal clearance (ml/min)	Clearance ratio (Cl_x/Cl_{inulin})
2′-Deoxyadenosine or dAdo	1.05 ± 0.05	0.360 ± 0.041	0.342 ± 0.036[a]	1.41 ± 0.13
Adenosine or Ado	13.55 ± 0.57	0.524 ± 0.144	0.046 ± 0.012[b, c]	0.20 ± 0.07
Inulin-(^{14}C)-carboxylic acid	4.34 ± 0.10[d]	1.074 ± 0.135	0.246 ± 0.026	–

Renal clearance measurements in male AKR mice were performed as described in Table 4. Methazolamide (25 mg/kg) was administered IP 10 min after DCF treatment. This treatment increased the urine pH from 6.0–6.5 to greater than 8.0. The values shown are the mean ± SE of four determinations in each case

[a] Significantly greater than the inulin clearance, $P < 0.025$, Student's paired t-test

[b] The renal clearance of Ado was corrected for the plasma binding (17%) of this nucleoside in mice (see Table 3)

[c] Significantly less than the inulin clearance, $P < 0.005$, Student's paired t-test

[d] Estimated 'midpoint' value for plasma inulin concentration (see Table 4)

clearance of Ado and inulin were measured in mice which had been treated with methazolamide, a carbonic anhydrase inhibitor. The carbonic anhydrase inhibitors are a class of agents that cause an alkaline diuresis by increasing renal excretion of bicarbonate [25]. The renal clearance of dAdo in methazolamide-treated mice was significantly greater than that of inulin (Table 6) and was not different from the clearance observed in control mice (Table 4). Similarly, Ado and inulin clearances values were not markedly altered by urine alkalinization with methazolamide (Tables 4 and 6).

Discussion

Apparent active renal transport processes for dAdo and Ado were observed in humans in whom a genetically or drug-induced ADA deficiency occurred (Tables 1 and 2). We have previously reported that the child with SCID : ADA^- excreted large amounts of dAdo in comparison to the amounts of Ado in his urine [10]. Similarly, other workers have reported the presence of measurable levels of dAdo in urine in this [15, 22] and other patients [4, 7, 22, 23]. When renal clearances of dAdo and Ado are compared with the estimated or measured renal clearance of creatinine, apparent net secretory and reabsorptive processes become manifest (Table 1). Specifically, the renal clearance of dAdo is greater than that of Ado or that of creatinine, whereas the clearance of Ado is less than that of creatinine (Table 1). Similar observations concerning the renal handling of these purine nucleosides were made in cancer patients receiving an ADA inhibitor, DCF, in a phase I–II trial of this drug (Table 2). In patients having normal levels of ADA activity, it is difficult to measure dAdo and Ado in plasma and urine, since these compounds are at or below the detectable level for the fluorescent HPLC method (Table 2). The renal clearances of dAdo and Ado are not influenced by binding to plasma proteins, since less than 5% binding was observed at or near the plasma concentrations attained in vivo after DCF treatment (Tables 2 and 3). The renal clearance of adenine could not be accurately determined, since plasma and urine levels of this substance were quite variable (Table 2). However, the low levels of adenine in plasma and urine indicate that dAdo was chemically stable in the extracts of plasma and urine during sample preparation and analysis [22].

Since the biochemical alterations and the renal handling of dAdo and Ado are similar in the SCID : ADA^- patient and in patients receiving DCF, it appears that DCF treatment does not alter the renal transport processes for these compounds in humans. Therefore, we have employed DCF treatment of mice as an animal model to study the renal transport of Ado and dAdo. Similar to the effect of DCF on plasma dAdo in humans, there is a progressive increase in dAdo in plasma of mice receiving 5 mg DCF/kg (Fig. 4), a dose which produced profound and prolonged inhibition of red blood cell ADA activity. Without DCF treatment, concentrations of Ado in plasma were erratic whether plasma was obtained via heart puncture, tail vein, or retro-orbital sinus (unpublished data). Nevertheless, after DCF treatment, dAdo and Ado levels were relatively constant during an interval in which renal clearance measurements were performed (Fig. 4 and Table 4). The renal clearance of dAdo in mice exceeded that of inulin about two-fold, whereas Ado clearance was approximately half that of inulin. Thus, the renal handling of these purine nucleosides in mice is qualitatively similar to that in humans.

Additional experiments were possible in mice to further substantiate that renal secretion of dAdo occurs. Specifically, with radiolabeled dAdo it was possible to eliminate the kidney as a primary source of the dAdo observed in urine which was in excess of the filtered fraction (Table 5). Also, an experiment in which a carbonic anhydrase inhibitor was used to alkalinize the urine indicated that the apparent secretion was not due to simple ion-trapping [16] in an otherwise acid urine (Table 6).

The above observations in humans and in mice suggest that the mammalian kidney has active transport mechanisms for dAdo and Ado, although the vectorial aspects of these putative transepithelial transport systems are in opposite directions. If such transport processes indeed exist, it is reasonable to propose that the processes are very selective, since dAdo and Ado are very similar structurally. The only structural difference relates to the presence or absence of a hydroxy group in the 2′-position of the furanosyl moiety. To our knowledge, these are the first indications of active transport systems for purine nucleosides in a mammalian tissue. The facilitated diffusion (metabolically independent) mechanism for the transmembrane transport of such compounds has been extensively studied [18]. Further investigations will be required to elucidate the transport mechanisms involved and to determine their relationships to other classic transepithelial transport systems such as the organic cation and anion secretory systems, glucose and ion reabsorption, etc.

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