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The Pharmacologic Fate of the Antitumor Agent 2-Amino-1,3,4-thiadiazole in the Dog

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Summary. *Anticipating the renewed clinical trial of the antitumor agent 2-amino-1,3,4-thiadiazole (AT, NSC-4728), we have studied the pharmacologic fate of AT-5-^{14}C in beagle dogs. The drug was only minimally metabolized in 5 h; the radioactivities in the urine, and particularly in the plasma, resided almost entirely in unchanged AT. In four dogs, after IV administration of AT-5-^{14}C at 10 mg/kg (200 mg/m^2), 150–200 μCi per animal, the terminal plasma $t_{1/2}$ of AT was 13 h, and less than 10% of the administered dose appeared in the urine in 5 h. When the dose was raised to 25 mg/kg (500 mg/m^2) in four dogs, the average plasma $t_{1/2}$ of AT was 10 h; the 5-h cumulative excretion of the agent was 9% of the dose in the urine, 0.3% in the bile, and 0.1% as $^{14}CO_2$ in the expired air. The average total clearance of AT was 0.70 ml/kg/min. Drug concentrations in the cerebrospinal fluid were 95% of those in the plamsa at semi-steady state. At autopsy 5 h after dosing, more than 75% of the administered radioactivity was retained in the body, the highest amounts in terms of micrograms per gram of wet tissue being in the liver, kidney, lung and stomach. No appreciable changes in AT pharmacokinetics were evident upon simultaneous IV administration of allopurinol at 30 or 60 mg/kg. AT was less than 7% bound to dog plasma protein at 25° C in concentrations of 12–100 μg/ml.*

Introduction

The antitumor activities of 2-amino-1,3,4-thiadiazole (AT, NSC-4728) were demonstrated in several experimental tumor systems more than 20 years ago [7]. The clinical usefulness of this agent is limited because of stomatitis and hyperuricemia. Unrelated to nucleic acid catabolism, the hyperuricemia could be reversed with nicotinamide, which unfortunately also abolished the antitumor effects [2]. Interest in AT is currently being revived because of the possible prevention of the clinical hyperuricemia with allopurinol without, however, diminishing its anticancer activity. Anticipating its renewed clinical trial at our institution, we have studied the pharmacologic fate of AT-5-^{14}C in the beagle dog. This report summarizes our finding.

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Materials and Methods

Drugs and Chemicals

AT and AT-5-^{14}C (specific activity 6.5 mCi/mmol, 99% pure chemically and radiochemically) were kindly supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Allopurinol sodium for parenteral administration was a product of Burroughs Wellcome Company. All chemicals and reagents were purchased from regular commercial sources. For liquid scintillation counting, we used a xylene-based phase-combining system (PCS) available from Amersham Corp., Arlington Heights, IL. Rapid tissue digestion was accomplished with a commercial solubilizer, Soluene-350, purchsed from Packard Instrument Co., Downers Grove, IL.

Radiochemical Techniques

Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer model 2650; quenching was corrected by the external standard channels ratio method; for ^{14}C the counting efficiency was about 90%. Plasma or urine (0.2 ml) was counted in 11 ml PCS. Paper and thin-layer chromatograms were scanned for radioactivity as previously described [4].

Tissues were thoroughly rinsed in cold 0.9% NACl and blotted dry; each 100-mg specimen was digested with 1 ml Soluene-350 at 50° C for 4 h. The homogeneous solution was cooled to room temperature, mixed with 0.2 ml isopropanol, and decolorized with 0.2 ml 30% hydrogen peroxide. The solution was

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left standing at room temperature for 1 h with occasional gentle swirling. The radioactivity was determined after the addition of 11 ml PCS.

Chromatography

Chromatography was conducted in three systems; all solvent compositions are in v/v. (A) Whatman no. 1 paper, *n*-butanol-acetic acid-water (5 : 3 : 2), descending. (B) Brinkmann silica gel 60F-254 plate, isopropanol-acetic acid-H_2O (4 : 1 : 1), ascending. (C) The same plate, *n*-butanol-acetic acid-ethyl acetate-H_2O (1 : 1 : 1 : 1). In these systems the R_f values of AT are 0.71, 0.85, and 0.81, respectively. AT is stable in these solvents, and is neither light- nor heat-sensitive.

Dogs

Beagle dogs, two male and two female in each dose group, 9–12 kg, were lightly anesthetized with pentobarbital. AT-5-^{14}C dissolved in sterile saline, 25 mg/ml, was administered IV by the femoral vein in about 10 min; each animal received 10 or 25 mg/kg (200–500 mg/m^2), 150–200 μCi total. The dose was chosen to correspond to that of our current phase I clinical trial of AT. Blood was collected at intervals from the opposite femoral vein upon completion of drug administration, heparin being used as an anticoagulant. The plasma was separated from cells by centrifugation of the blood at 12,000 *g* for 10 min in a Sorvall RC2-B centrifuge. Urine was collected with an indwelling Foley catheter; the bladder was flushed with 0.9% NaCl each time, and the flushing combined with the respective urine specimen. The cerebrospinal fluid (CSF) was sampled by cisternal puncture, and the bile by cannulation of the common bile duct. By means of an endotracheal tube, the expired air was allowed to pass through a trap containing 250 ml phenethylamine-methanol (1 : 1) for the absorption of $^{14}CO_2$. The tissue distribution of radioactivity was determined in three dogs: two received 10 mg/kg and one received 25 mg/kg. The dogs were sacrificed by exsanguination 5 h after drug administration, and the major organs were dissected, removed, and weighed. The tissue radioactivity was measured as described above.

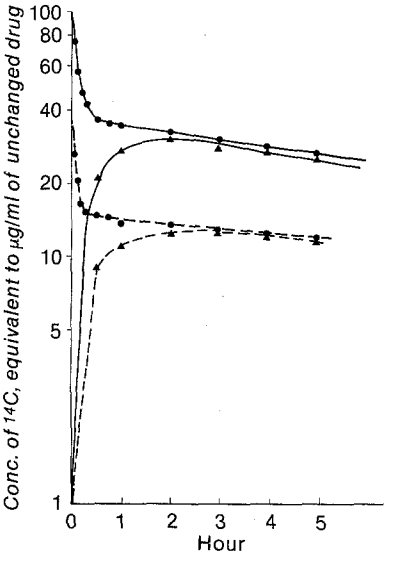

Fig. 1. Average plasma and CSF levels of ^{14}C in beagles after AT-5-^{14}C IV. ———, 25 mg/kg (4 animals); — — —, 10 mg/kg (3 animals); ●, plasma level; ▼, CSF level

Protein Binding

Binding of AT to plasma protein was determined by a modified ultrafiltration technique with an Amicon Centriflo® membrane-cone type CF 25, which permits the passage only of molecules of less than 25,000 molecular weight. Plasma solutions of AT were ultrafiltered through this membrane-cone after centrifugation at 500 *g* for 30 min.

Results

In four dogs, after IV administration of AT-5-^{14}C, 10 mg/kg, the average terminal plasma $t_{1/2}$ of total ^{14}C was 13 h (Fig. 1 and Table 1). However, since we were able to show subsequently that AT was minimally metabolized, and that the plasma radioactivity resided 98%–99% in the unchanged drug during the first 5 h (Fig. 2), the plasma total radioactivity was therefore completely equivalent to AT concentration. The apparent volume of distribution of AT was 319 mg/kg, similar to the sodium space in

Table 1. Pharmacokinetics of AT-5-^{14}C in the beagle

Dose		No.	$t_{1/2}$		Vd ml/kg	Total plasma clearance ml/kg/min	% Excretion in 5 h		
mg/kg	mg/m^2		Initial min	Term. h			Urine	Bile	$^{14}CO_2$
10	200	4	4	13	319	0.6	9.2	ND	ND
25	500	4	5	10	293	0.8	9.2	0.3	0.1
25[a]	500	2	5.3	13	450	0.7	6.9	ND[c]	ND
25[b]	500	1	4.1	13	527	0.8	14.0	ND	ND

[a] With allopurinol, 60 mg/kg
[b] With allopurinol, 30 mg/kg
[c] ND, not done

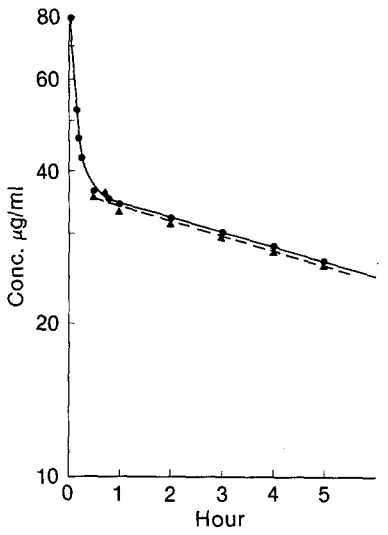

Fig. 2. Average plasma levels of ^{14}C and AT in four beagles after 25 mg/kg AT-5-^{14}C/kg IV. ●——●, total ^{14}C; ▲——▲, unchanged AT

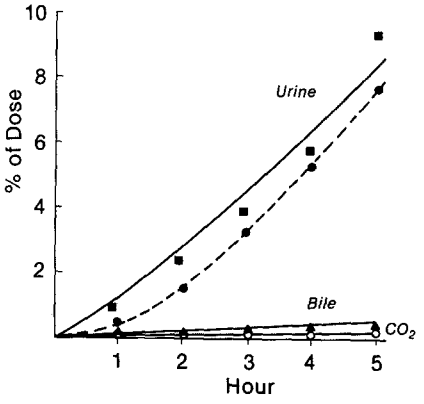

Fig. 3. Average cumulative excretion of ^{14}C in beagles after administration of AT-5-^{14}C IV. ———, after 25 mg/kg (4 animals); — — — —, after 10 mg/kg (3 animals)

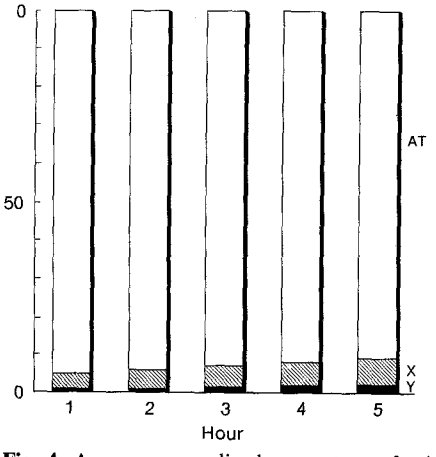

Fig. 4. Average normalized percentage of urinary metabolites in four beagles after 25 mg/kg AT-5-^{14}C/kg IV

the dog. After a higher dose, namely 25 mg/kg, the terminal plasma $t_{1/2}$ of AT was 10 h in four dogs, and the apparent volume of distribution remained in the range of the sodium space, that is 293 ml/kg (Fig. 1 and Table 1). The average total clearance of AT was 0.8 ml/kg/min. Regardless of dose, AT penetrated the blood-brain barrier readily (Fig. 1); from 2 to 5 h after drug administration, plasma and CSF drug concentrations were virtually identical.

The urinary excretion of AT was slow, about 9% of the dose in 5 h (Fig. 3 and Table 1). Accordingly, we collected the bile and expired air from the four dogs that received AT at 25 mg/kg. However, in 5 h, only 0.3% of the dose was found in the bile, and still less, 0.1% in the expired air as $^{14}CO_2$ (Table 1).

We studied the possible pharmacokinetic interaction between AT and allopurinol because such a drug combination has been advocated for the prevention of the undesirable hyperuricemia caused by AT [3]. To this end we administered allopurinol, 60 mg/kg to two animals and 30 mg/kg to another, together with AT IV at 25 mg/kg. In these three dogs, the average terminal plasma $t_{1/2}$ of AT was 13 h, and the 5-h cumulative excretion was 9%. However, the average apparent volume of distribution of AT was 476 ml/kg, considerably larger than that in the dogs that received AT alone (Table 1). But there was no significant alteration in the total clearance.

The metabolism of AT was negligible; 5 h after drug administration less than 2% of the plasma and CSF radioactivity was in AT metabolites. This is consistent with our observations that in the first-hour urine, the percentage of metabolites was about 5%, and in the fifth-hour urine it was less than 10%; Fig. 4 shows the average normalized percentage distribution of AT and metabolites in the 5-hourly urine specimens of four dogs. Moreover, in the liver and kidneys, less than 4% of the total radioactivities was in AT metabolites. We have detected two radioactive metabolites by paper chromatography in solvent system A. The dominant metabolite was X, R_f 0.40; it imparted a positive color test for primary amines with 4-dimethyl-aminobenzaldehyde. The other metabolite, Y, R_f 0.18, never exceeding 2% in the urine, gave no such color test for primary amines. These were not further studied.

The average tissue distribution of AT-5-^{14}C 5 h after IV administration of the agent is summarized in Table 2. In terms of micrograms of drug per gram of wet tissue, the urinary bladder, thyroid, stomach, lung, kidney, and liver showed the highest drug concentrations; however, the results in the urinary bladder and thyroid were based on one dog only. On the other hand, in terms of percentage of the administered dose, most AT was in the muscle and

Table 2. Tissue distribution of ^{14}C 5 h after IV administration of AT-5-^{14}C in the beagle

Tissue	μg/g wet tissue		% of dose	
	10 mg/kg[a]	25 mg/kg[c]	10 mg/kg[a]	25 mg/kg[b]
Urinary bladder	17.2[b]	ND[d]	0.1[b]	ND
Thyroid	16.8[b]	ND	< 0.1[b]	ND
Stomach	15.2	63.6	1.4	2.5
Lung	14.2	36.9	1.3	2.8
Kidney	13.9	29.5	0.8	0.6
Liver	13.1	32.1	4.2	3.3
Spleen	11.3	16.1	0.7	0.5
Muscle	11.1	25.5	51.0	47.0
Brain	11.1	25.8	0.7	0.7
Small intestine	10.8	21.2	3.6	2.0
Large intestine	10.2	20.1	0.7	0.4
Heart	9.9	25.9	0.8	0.9
Diaphragm	9.9	22.2	0.4	0.4
Pancreas	9.8	18.7	0.3	0.2
Skin	8.7[b]	ND	13.9[b]	ND
Tendon	5.2[b]	ND	ND	ND
Fat	2.2	3.6	ND	ND
			80.0	61.3

[a] Average of three dogs except where noted
[b] One dog
[c] Average of two dogs
[d] ND, not done

skin (one dog only), and about 4% in the liver and small intestine.

In concentrations of 12.5–100 μg/ml, AT was about 5% bound to dog plasma protein, and 10% to human plasma protein, probably albumin in both species. Assuming one binding site per albumin molecule, and an average albumin concentration of 5×10^{-4} *M*, the association constants of the AT-albumin complexes at 25° C are 2.5×10^2 per mole and 1.3×10^2 per mole for human and dog plasma, respectively.

Discussion

Since AT was only minimally metabolized during the 5-h experimental period, the radioactivities measured were essentially those due to the unchanged drug. Although a relatively small molecule with a molecular weight of 101, freely soluble in water, and not extensively bound to plasma protein, its extrapolated apparent volume of distribution suggests that it is largely confined to the extracellular space, like the sodium cation. On the other hand, our studies indicate that AT readily penetrates the blood-brain barrier. The drug is comparatively slowly cleared from the plasma, with the kidney and the liver contributing little to the total clearance. This is hardly surprising, since within 5 h of drug administration most of the dose is taken up by the skeletal muscle and other tissues. In other words, binding to various tissues must be a major route of elimination of AT from the plasma.

The observation that AT is excreted in the urine principally unchanged in the first 5 h is reminiscent of similar findings with acetazolamide, another 1,3,4-thiadiazole derivative that is also excreted mainly intact [5]. Apparently the 1,3,4-thiadiazole ring is resistant to in vivo biotransformation. One may recall that the herbicide tebuthiuron [1-[5-(1,1-dimethylethyl)-1/,3,4-thiadiazol-2-yl]-1,3-dimethylurea] is extensively metabolized in many species to a number of products all retaining the ring structure [6].

After completion of this work, the distribution and metaolism of AT in mice, dogs, and monkeys was reported by others elsewhere [1]. These authors also noted that in the dog AT was excreted unchanged through 12 h, but metabolism became significant through 24 h. However, they described that plasma AT and metabolites concentrations decreased exponentially with a half-life of 6 h. The reason for the discrepancy between their results and ours remains obscure, but they only studied two animals (whereas we studied five).

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