On the Bioavailability of Oral and Subcutaneous 2-Chloro-2′-Deoxyadenosine in Humans: Alternative Routes of Administration

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Purpose: **The antimetabolite 2-chloro-2′-deoxyadenosine (CdA) is a promising alternative to alkylating agents for the treatment of lymphoproliferative disorders. Its use, however, is hampered by the need for intravenous (IV) administration. The aim of the present study was to determine the bioavailability of subcutaneously (SC) and orally administered CdA, and to establish an oral dose of CdA that could supersede IV administration.**

Patients and Methods: **A previously developed high-performance liquid chromatography method was used for the determination of plasma CdA concentrations in 13 patients. Ten patients were treated on alternate days with 0.14 mg/kg/d CdA as a 2-hour IV infusion or by a SC injection. Three of these patients were also given 0.14 mg/kg CdA orally in enteric-coated capsules. Ten patients were administered CdA orally that was dissolved in phosphate-buffered saline (PBS) after treatment with 20 mg omeprazole 1 and 6 hours before the administration of CdA.**

Results: **The bioavailability of SC CdA was 102% ± 28% (mean ± SD), and the bioavailability of CdA administered in enteric-coated capsules was 19%, 24%, and 60%. In the three patients who were given 0.14 mg/kg orally dissolved in PBS, the bioavailability was 48% ± 8%, whereas in the seven patients who received 0.28 mg/kg, the bioavailability was 55% ± 17%. In the 10 patients who were treated with the CdA solution orally, the coefficients of variation of the areas under the curve (AUCs) after oral and IV administration were similar. Thus, oral administration did not add to the interindividual variability.**

Conclusions: **We conclude that orally administered CdA can supersede IV infusion if the dose is doubled. SC administration gives a high peak concentration of short duration with an AUC identical to that of IV infusion. Thus, SC injection can also be used as an alternative to IV infusion.**

J Clin Oncol 10:1514-1518. © 1992 by American Society of Clinical Oncology.

UNTIL RECENTLY, the primary treatment of choice for low-grade non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) has been an alkylating agent, mostly chlorambucil, with or without prednisone. However, the reports by Keating et al[1,2] have shown that the antimetabolite fludarabine is a promising alternative.

2-chloro-2′-deoxyadenosine (CdA), another halogenated purine analog, was synthesized more than 20 years ago.[3] Its clinical activity in lymphoproliferative disorders has been delineated by Beutler et al.[4] Its outstanding activity in hairy cell leukemia,[5] with an 85% complete remission rate,[4] triggered a large interest for this antimetabolite. The activity in CLL[4,6] and NHL[4,7] has been less impressive, although responses have been observed in pretreated patients with a highly refractory disease. We have also recently shown that CdA may be active in patients with CLL that is resistant to fludarabine.[8] Therefore, phase III trials that compare the effects of chlorambucil, fludarabine, and CdA are needed greatly.

The pharmacokinetics of CdA after intermittent and continuous intravenous (IV) infusions have been delineated[9,10] using a recently developed high-performance liquid chromatography method.[11] The aim of the present study was to determine the bioavailability of subcutaneous (SC) and orally administered CdA and to establish an oral dose of CdA that could supersede IV administration.

PATIENTS AND METHODS

Thirteen patients with B-cell CLL and low-grade NHL were treated with CdA for 5 consecutive days. On the first 3 days, all patients were administered CdA that alternated as a 2-hour IV infusion, SC, or orally with alternate order between patients. IV infusions and SC injections of CdA were given in a dose of 0.14 mg/kg, whereas oral CdA was administered in three different ways. Three patients were given 0.14 mg/kg CdA in enteric-coated capsules to prevent hydrolyzation of the compound in the acid environment of the stomach. Another 10 patients were given CdA orally in the same phosphate-buffered saline (PBS) solution that was used for IV and SC administration. Three patients received 0.14 mg/kg, whereas seven patients received 0.28 mg/kg. Because

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Submitted February 10, 1992; accepted June 10, 1992.

This study was supported by grants from the Swedish Cancer Foundation, the Swedish Medical Research Council, and the Jenny Foundation.

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0732-183X/92/1010-0003$3.00/0

CdA is not stable in an acid environment, 20 mg omeprazole was given 6 and 1 hours before the CdA to reduce the acid secretion in the stomach in these 10 patients. All patients were fasted from at least 3 hours before and until 3 hours after oral administration. The patients had given their informed consent to participate in the study, which was approved by the local ethics committee at Huddinge Hospital and the Swedish Drug Product Agency.

Preparation of the Drug

CdA was synthesized by Dr Kazimierczuk,[12] and a sterile, pyrogen-free solution (2 mg/mL) in PBS (pH = 7.4) was prepared. Some of the CdA was encapsulated in enteric-coated capsules that contained 2 or 3 mg CdA. The capsules were coated with methacrylic acid copolymere type A (Eudragit L; Röhm Pharma, Weiterstadt, Germany) and diethylphthalate. A disintegration test was performed according to the standards of the European pharmacopoeia.[12]

The capsules were stable in 0.1 N hydrochloric acid for more than 2 hours, and disintegrated in less than 7 minutes in a phosphate buffer with pH 6.8. All doses to patients who received capsules were approximated to multiples of 1 mg. CdA was diluted in 500 mL saline for IV infusion, whereas the 2-mg/mL solution was used directly for SC injection (in the abdominal adipose tissue) or oral administration.

Blood Sampling Procedure

Five to 10 mL of blood was taken from a separate peripheral IV access into heparinized tubes 15, 30, and 45 minutes and 1, 1.5, 2, 3, 3.5, 4, 5, 6, 9, 12, 16, and 22 hours after CdA administration. During IV administration, samples were also taken at 30 minutes and 1, 1.5, and 2 hours during infusion and 5 minutes after the end of infusion. The tubes were immediately put into ice water, and the plasma was collected by centrifugation (7 minutes; 550 × *g*; 4°C) and frozen at −20°C until analysis.

Assay Method

The plasma concentration of CdA was determined with reversed-phase high-speed high-performance liquid chromatography that used 6-([1-methyl-4-nitro-5-imidazolyl]thio)guanine (the 2-amino analog of azathioprine, Guaneran, was a gift from Dr Gertrude Elion, Wellcome Foundation, Research Triangle Park, NC) as internal standard.[11] CdA and the internal standard were extracted from the plasma with ethylacetate in silanized glass tubes; the detection limit was 1 nmol/L.

Pharmacokinetic and Statistical Evaluation

The pharmacokinetic analysis was performed using a nonlinear estimation program PC-Nonline (Statistical Consultants Inc, Lexington, KY). After oral and SC administration, the data in all cases were fitted best to a two-compartment model. Most of the data obtained from patients who were treated IV could also be fitted to a two-compartment model; only five patients were fitted best to a three-compartment model. All data points for the second and third dose were corrected for residual concentrations from the previous dose, after extrapolation of the terminal phase. In all patients who were treated with oral CdA solution, the area under the curve (AUC) and its coefficient of variation (CV) were calculated after normalization for dose-assuming linear pharmacokinetics.

RESULTS

The pharmacokinetic determinants after oral, SC, and 2-hour IV infusion of CdA are listed in Table 1. The bioavailability of CdA administered in an acid-resistant capsule was 34% ± 22% compared with 48% ± 8% when CdA was administered orally as a PBS. When the dose of CdA administered orally was doubled, the bioavailability was 55% ± 17%. When administered SC, its bioavailability was close to 102% ± 28%. The pharmacokinetic profile after the three modes of administration is shown in Fig 1. The highest mean peak concentration, 318 ± 91 nmol/L, was observed in the patients who were treated with SC injection, whereas IV infusion yielded a peak concentration of 169 ± 90 nmol/L. After the administration of 0.28 mg/kg orally, the peak concentration was 196 ± 149 nmol/L, whereas after the 0.14 mg/kg dose it was only 53 ± 6 nmol/L. The time to peak was shorter after SC injection (0.34 ± 0.13 hours) compared with the oral route (1.17 ± 1.04 hours), and the pharmacokinetic profile of the latter resembled the IV 2-hour infusion more than did the SC injection.

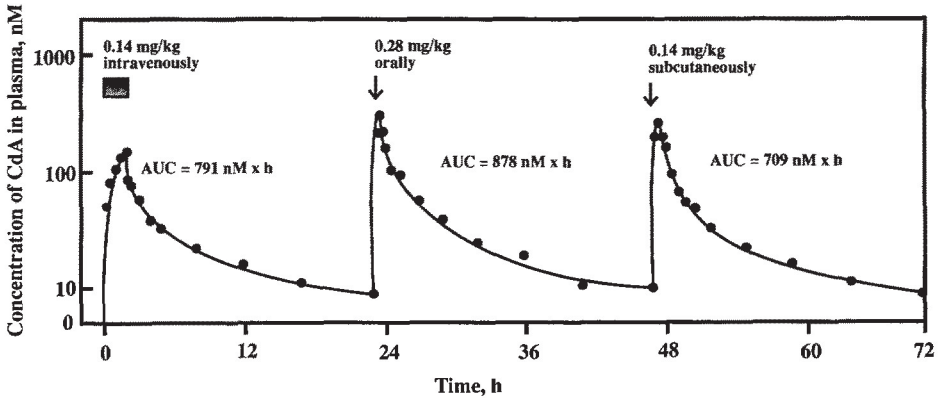

Fig 1. The plasma concentration of CdA after IV, SC, and oral administration in patient no. 7. The oral dose of CdA was 0.28 mg/kg. The doses are indicated by (■) IV infusion or (↓) SC or oral.

Table 1. Pharmacokinetic Parameters of CdA After IV 2-Hour Infusion, SC Injection, and Three Schemes of Oral Administration

	Auc (O∞)					Bioavailability (%)				Tmax		Cmax		
							Oral							
Patient	IV (nmol/L × h)	SC (nmol/L × h)	Capsules (nmol/L × h)	Solution 0.14 mg/kg (nmol/L × h)	Solution 0.28 mg/kg (nmol/L × h)	SC	Capsules	0.14	0.28	SC (hours)	Oral (hours)	IV (nmol/L)	SC (nmol/L)	Capsules (nmol/L)
1	1,069	746	201			70	19			0.37	2.75	432	371	27
2	769	1,056	459			137	60			0.33	2.97	208	445	99
3	1,295	805	308			62	24			0.50	0.43	134	239	42
4	827	831		323		100		39		0.25	3.10	203	312	
5	1,084	1,137		531		105		49		0.30	0.80	128	305	
6	619	937		346		151		56		0.22	1.11	134	358	
7	709	791			878	112			62	0.25	0.30	140	269	
8	709	590			493	83			35	0.17	0.55	152	412	
9	399	422			661	106			83	0.42	0.57	68	128	
10	745	674			953	90			64	0.60	0.30	167	348	
11	394				380				48		0.70		89	
12	412				280				34		1.18		126	
13	866				1,025				59		0.50		221	
Mean	761	799	323	400	667	102	34	48	55	0.34	1.17	169	318	56
SD	276	212	130	114	294	28	22	8	17	0.13	1.04	90	91	38
CV	0.36	0.27	0.40	0.29	0.44	0.27	0.65	0.18	0.32	0.39	0.89	0.53	0.29	0.68

Although some intraindividual variability was observed, the terminal half-life ($t_{1/2}$) of CdA was independent of the mode of administration. Because of the long elimination phase, there was a spillover from day to day (median, 10.4%), which was considered in the calculation of AUCs for the second and third dose (see Patients and Methods). The interindividual variability of the AUC after oral solution (CV, 0.38) was not greater than the IV AUC (CV, 0.36) and was only slightly larger than that of the SC administration (CV, 0.27). After the administration of CdA in enteric-coated capsules, the interindividual variation in bioavailability and AUC was somewhat larger (CV, 0.54).

Patients tolerated SC and oral administration very well. No local tenderness, rash, or other symptoms were observed at the site of SC injection in any of the patients. No diarrhea or dyspepsia was experienced after oral administration.

DISCUSSION

The present study demonstrates that CdA can be administered SC or orally. Although the bioavailability of CdA administered orally is only approximately 50%, an AUC and a pharmacokinetic profile that resembles that of a 2-hour IV infusion can be obtained if the dose is doubled.

Surprisingly, the interindividual variability of AUC after the oral solution was not greater compared with that of the IV infusion. Although it was compensated for, the spillover from day to day might make the calculations for individual patients somewhat uncertain. The median spillover was only 10.4%, however, and because the order in which the different modes of administration were given was altered, the mean values were reliable. Despite similar AUC values, the peak concentration was considerably higher after SC injection than after IV infusion. Therefore, it cannot be concluded directly from these data that the two modes of administration were bioequivalent. However, in vitro investigations have shown that there seemed to be no saturability in the rate of phosphorylation of CdA at drug concentrations less than 10 μmol/L,[14] which was considerably higher than the plasma concentrations obtained after SC injection. The t½ of intracellular CdA nucleotides was approximately 24 hours.[10] When taken together, this suggested that the time during which the plasma concentration of CdA was above a certain threshold was not important. Therefore, we suggest that SC injection, as a more convenient mode of administration, can also replace IV infusion.

Beside hairy cell leukemia, CdA is most efficacious in the treatment of CLL and low-grade NHL. In the early stages, these diseases usually are treated with oral alkylating agents that do not require hospitalization. The initial response rate of this treatment is fair, but trials with the antimetabolites fludarabine and CdA suggest that there are prospects for a better treatment outcome. So far these new agents have been administered IV. This requires hospitalization of patients for 5 to 7 days per month for longer time periods, which must

Table 1. Pharmacokinetic Parameters of CdA After IV 2-Hour Infusion, SC Injection, and Three Schemes of Oral Administration (Cont'd)

Cmax		$t_{1/2\alpha}$			$t_{1/2\beta}$			Vd				
Solution 0.14 mg/kg (nmol/L)	Solution 0.28 mg/kg (nmol/L)	IV (hours)	SC (hours)	Oral (hours)	IV (hours)	SC (hours)	Oral (hours)	IV (L/m²)	SC (L/m²)	Oral (L/m²)	Cl (1/h × m²)	Order of Dose
		1.63	1.96	0.98	12.1	16.7	11.5	46.3	37.7	66.8	18.7	IV, oral, SC
		2.33	2.57	1.92	13.9	17.3	7.7	41.3	57.0	92.1	23.9	Oral, IV, SC
		0.41	1.82	0.23	21.8	17.3	7.1	44.7	26.3	50.3	13.5	Oral, SC, IV
52		0.37	0.80	1.84	4.5	6.6	17.3	31.0	52.7	90.5	21.7	Oral, SC, IV
59		0.37	2.32	0.83	12.9	18.7	10.7	49.0	46.7	92.3	15.7	SC, oral, IV
48		0.42	2.30	0.77	6.5	26.5	11.3	52.7	45.9	97.6	30.6	SC, oral, IV
	226	0.37	0.71	0.89	9.1	11.2	9.2	83.0	68.3	91.0	28.2	IV, oral, SC
	98	0.24	1.01	0.85	6.4	5.6	10.0	32.3	32.3	104.1	29.4	SC, oral, IV
	119	0.54	0.53	0.45	7.8	8.1	10.1	111.9	106.7	128.7	40.9	IV, SC, oral
	375	0.45	0.64	0.95	9.6	4.9	7.6	40.2	55.0	40.2	25.3	SC, oral, IV
	75	0.76		1.57	8.0		17.3	79.9		135.2	35.2	IV, oral
	59	0.66		0.84	12.0		7.1	48.6		62.9	32.1	IV, oral
	422	0.57		0.83	4.8		8.7	35.8		38.2	21.8	IV, oral
53	196	0.70	1.47	1.00	9.9	13.3	10.4	53.6	52.9	83.8	25.9	
6	149	0.60	0.80	0.50	4.6	7.1	3.4	23.7	22.6	30.7	7.8	
0.11	0.76	0.86	0.55	0.50	0.47	0.53	0.33	0.44	0.43	0.37	0.31	

Abbreviations: Tmax, time of maximum concentration; Cmax, maximum concentration; Vd, volume of distribution; Cl, clearance.

be considered a major impairment of the quality of life. An alternative mode of administration of these agents, therefore, is required. We present the necessary pharmacokinetic data for oral CdA treatment. Because the bioavailability of oral fludarabine is approximately 80%,[15] it will be possible to compare fludarabine and CdA under equal conditions by using oral preparations (with or without an alkylating agent as control treatment).

Questions about the bioavailability of CdA still need to be answered. We do not yet know the effect of food intake, whether antacids improve bioavailability, or whether the absorption of orally administered CdA is dose-dependent. These matters are under investigation and will be reported elsewhere.

ACKNOWLEDGMENT

Mats Johansson, Gunh Mellström, and Hans Sellander at the Center for Chemical Reference Substances at the National Corporation of Swedish Pharmacies are greatly acknowledged for the production of enteric-coated capsules, and Gudrun Ekberg at the Department of Pharmacy at Huddinge Hospital for the formulation and pharmaceutical handling of the drug.

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