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On the bioavailability of 2-chloro-2′-deoxyadenosine (CdA)

The influence of food and omeprazole

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Summary. The pharmacokinetics of oral CdA (0.24 mg/kg) was studied in 4 patients (1 with hairy cell leukaemia and 3 with B-cell chronic lymphocytic leukaemia) to determine any effect of food and fasting with and without omeprazole.

Food intake did not significantly influence the bioavailability of CdA (42% after food intake vs 46% while fasting) but it did reduce the maximum plasma concentration (C_{max}) by 40%; 83 compared to 116 nM while fasting. The time to reach maximum concentration (t_{max}) was delayed about 0.8 h after food intake. Pretreatment with omeprazole did not significantly influence the bioavailability of CdA (51% vs 46% without), or the interindividual variability in bioavailability in the fasting state (C. V. 0.26 with and C. V. 0.27 without).

In conclusion, there was a small, though not statistically significant reduction in the bioavailability of CdA after food intake. Omeprazole did not significantly improve the bioavailability of CdA.

Key words: 2-Chloro-2′-deoxyadenosine (CdA); omeprazole, food, pharmacokinetics, bioavailability

The antimetabolite 2-chloro-2′-deoxyadenosine(CdA) is a purine analogue which is resistant to adenosine deaminase, probably due to protonisation at the N-7 position [1]. It leads to accumulation of DNA strand breaks, thus activating poly(ADP)ribosylation, depletion of NAD, and apoptosis, causing cell death both in resting and dividing cells [2, 3].

CdA is the drug of choice for the treatment of hairy cell leukaemia (HCL) and it is a promising drug in the treatment of lymphoproliferative disorders, such as chronic lymphocytic leukaemia, non-Hodgkin's lymphomas [4–7] and relapsed acute myeloid leukaemia [8]. Patients with low-grade malignant lymphoproliferative diseases are generally treated as out-patients, and more convenient modes of administration of CdA than the i.v. route now used are needed.

We have recently delineated the pharmacokinetics of CdA in man after oral, subcutaneous and, intravenous administration [9, 10]. Those studies suggested that subcutaneous administration would be bioequivalent to i.v. infusion. The oral bioavailability of CdA was approximately 50%, but an AUC similar to that of an i.v. infusion could be achieved by increasing the dose by 100%. Due to the possibility that the acid environment of the stomach might cause degradation of CdA [11], omeprazole was co-administered when CdA was given orally to fasting patients.

The present study was undertaken to determine whether food intake altered the bioavailability or the pharmacokinetics of CdA. We also wanted to discover whether omeprazole, a gastric antisecretory drug which inhibits acid secretion by inhibition of H^+, K^+-ATPase, could improve the bioavailability of oral CdA.

Materials and patients

Drug synthesis

CdA used in this study was synthesised by Dr. Z. Kazimierczuk (Foundation for the Development of Diagnostic and Therapy, Warsaw, Poland [12]). The molar extinction coefficient of CdA was 15000. A solution (2 mg·ml^{-1}) of CdA in saline (9 mg·ml^{-1}) was prepared in the Huddinge hospital pharmacy and was shown to be sterile and pyrogen-free according to the standards of the European pharmacopoeia.

Patients and treatment

Four male patients, one with HCL and three with B-cell chronic lymphocytic leukaemia, participated in the study after giving prior informed consent (Table 1). The study was approved by the local Ethics Committee at Huddinge Hospital and by the Swedish Medical Products Agency. CdA was administered on five consecutive days. The s.c. and i.v. (2 h infusion) dose on Days 1 and 2 was 0.12 mg·kg^{-1}. The oral dose was 0.24 mg·kg^{-1} administered in saline after a standard breakfast or after overnight fast. Any residual CdA was rinsed from the dosage cup with 10 ml phosphate buffered saline (pH = 7.4) and was swallowed. The standard breakfast consisted of aproximately 100 g bread, 20 g ham, 20 g cheese and 150 ml coffee. On Day 3 the patient fasted overnight, and omeprazole 20 mg (Losec®) was given orally 6 h and 1 h before the last oral dose of CdA to protect CdA from

Table 1. Characteristics of the patients

Patient	Sex	Age year	Weight kg	Height cm	Diagnosis	Order of dose
1	Male	55	87	180	CLL	− −, + −, + +, IV
2	Male	44	69	177	HCL	+ −, − −, + +, SC
3	Male	70	88	172	CLL	− −, + −, + +, SC
4	Male	60	83	171	CLL	− −, + −, + +, SC
Mean		57	82	175		
SD		10.8	8.8	4.2		

Not fasting, no omeprazole (− −); fasting, no omeprazole (+ −); fasting, omeprazole (+ +)

Table 2. Summary of pharmacokinetic parameters of 2-chloro-2′-deoxyadenosine (CdA) during fasting, non-fasting and concomitant administration of omeprazole

Patient	Parental route	t_{max} (h)			C_{max} (nmol·l^{-1})			AUC (nmol·h·l^{-1})				$t_{1/2}\beta$ (h)				CL (l·h^{-1}·m^{-2})	Bioavailability (%)		
		− −	+ −	+	− −	+ −	+	SC/IV	− −	+ −	+	SC/IV	− −	+ −	+		− −	+ −	+
1	i.v.	0.80	0.58	0.53	176	134	249	977	925	743	1044	16.5	21.6	17.9	12.8	34.4	47	38	53
2	s.c.	1.59	0.50	0.58	64	133	111	660	614	599	680	15.3	16.4	13.7	14.7	37.6	47	45	50
3	s.c.	1.55	0.53	0.77	38	59	113	701	397	501	666	14.5	12.6	13.3	11.7	35.4	28	36	48
4	s.c.	1.05	0.60	0.46	55	139	182	454	400	570	494	7.4	18.7	10.1	5.1	49.1	44	63	54
Mean		1.25	0.55	0.59	83	116	164	698	584	603	721	13.4	17.3	13.8	11.1	39.1	42	46	51
S.D.		0.39	0.05	0.13	63	38	66	215	249	102	231	4.10	3.80	3.20	4.17	6.79	9.11	12.29	2.75
C.V.		0.31	0.08	0.23	0.76	0.33	0.40	0.31	0.43	0.17	0.32	0.31	0.22	0.23	0.38	0.17	0.22	0.27	0.05
P		< 0.05			NS				NS				NS				NS		

Not fasting, no omeprazole (− −); fasting, no omeprazole (+ −); fasting, omeprazole (+ +)

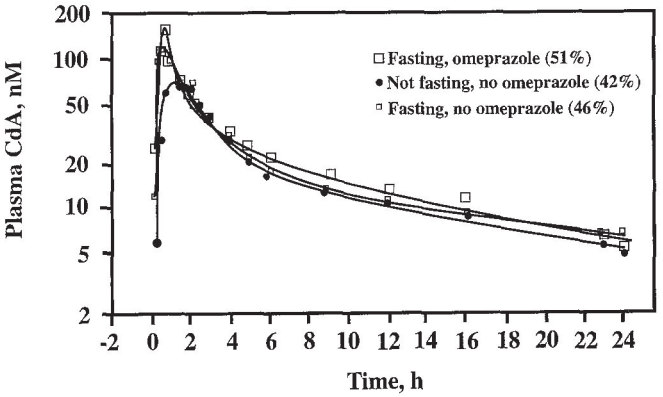

Fig. 1. The mean plasma concentration of CdA in 4 patients oral administration of 0.24 mg/kg CdA in three conditions. No fasting (●), fasting (□), fasting with concomitant omeprazole (□)

hydrolysis in the acidic environment. The patient was prohibited from drinking water for 3 h and eating for 5 h after CdA administration. On Days 4 and 5, CdA was given s.c. or i.v.

After drug administration, blood samples were collected from a separate peripheral vein into heparinised tubes immediately before drug administration and 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 18 and, 24 h afterwards. When administered s.c. or i.v., samples were also taken 5 and 10 min after administration. The samples were immediately put on ice, plasma collected by centrifugation (7 min, 550 × G, 4 °C) and stored at − 20 °C until analysis.

Plasma concentration assay

The plasma concentration of CdA was determined using a prefrom Dr. Gertrude Elion (Wellcome Foundation, Research Triangle Park, N.C.), was used as the internal standard and was added before extraction with ethyl acetate in silanized glass tubes. A C18, 3 µM column was used, with a mobile phase consisting of phosphate buffer, methanol, and acetonitrile (85:10:5, pH = 3.0) as the mobile phase at a flow rate of 1 ml·min^{-1}. The drug concentration was determined by UV-absorption. The inter-day variability was 8% at 5 nmol and 5% at 100 nmol. The limit of sensitivity was 1 nM.

Pharmacokinetic calculation

Plasma CdA concentration-time profiles were analysed by extended non-linear least-squares regression, using a commercially available program (Siphar, Société Simed, Creteil, France). The rate constant, the area under the plasma concentration versus time curve (AUC) and clearance were determined. The residual area from Day 1 was subtracted from the AUC of Day 2 etc. The AUC was also calculated using the trapezoidal rule and extrapolation to infinity using log-linear regression analysis of at least four points of the elimination phase. The results were in agreement with the model-dependent calculation. Clearance was calculated as dose/AUC. The bioavailability (f) of CdA in each patient was calculated as:

$$F = \frac{\text{AUC p.o.} \times \text{Dose s.c. or i.v.}}{\text{AUC s.c. or i.v.} \times \text{Dose p.o.}}$$

The observed maximum plasma concentration (C_{max}) and the time when it occurred (t_{max}) were tabulated for each patient and treatment.

Statistical analysis

Statistical analysis was carried out by comparison of the pharmacokinetic parameters between the fasting and non-fasting states, with and without omeprazole, using one way analysis of variance proce-

Results

The bioavailability of oral CdA and the calculated pharmacokinetic parameters are presented in Table 2. The disposition of CdA could best be described by a two-compartment model [8, 9, 10]. The pharmacokinetic profile of CdA administered orally during the non-fasting, fasting and fasting state in combination with omeprazole are depicted in Fig. 1. The individual plasma concentration-time profiles of CdA are shown in Figs. 2 a, 2 b, 2 c.

The administration of omeprazole 1 and 6 h before swallowing the dose did not have a significant influence on the bioavailability of CdA (51 and 46 % with and without omeprazole respectively). In a previous study [10] omeprazole was given to all patients who were treated with oral CdA fasting. Addition of data from that study to the present, showed that there was no difference in the interindividual variation in the bioavailability between the fasting state with and without concomitant omeprazole, and after food intake (C. V. 0.26 vs 0.27 vs 0.22). Concurrent food intake did show some influence on the rate of absorbtion, increasing the t_{max} (1.25 vs 0.55 and 0.59 h) and lowering the C_{max} (83 vs 116 and 164 nM). The bioavailability was also slightly but no significantly lower (42 vs 46 and 51 %) in this small cohort of patients.

It is noteworthy that the interindividual variability in t_{max}, C_{max} and AUC was less in the fasting state than after food intake (Table 2). The pharmacokinetic parameters, plasma clearance, volume of distribution and disposition constant were in agreement with previously reported values [9, 10].

Discussion

CdA is one of the most promising new drugs for the treatment of low-grade malignant lymphoproliferative diseases. Oral administration would simplify the treatment both for patients and physicians. We have recently shown that the bioavailability of CdA was about 50 % when administered to fasting patients and with omeprazole. The present study has provided further data on the bioavailability of CdA after food intake and after fasting with and without omeprazole. Since the bioavailability of omeprazole after a single dose is very low, patients were given two doses 6 and 1 h before CdA intake to ensure that acid secretion was inhibited [15].

There are several plausible explanations why the bioavailability of CdA was only 50 %. First, its low pKa (1.4) [1] implies that most of the drug will be ionized in the alkaline environment of the gut, which will tend to result in incomplete absorption. Second, there might be some first-pass loss in gut wall/liver. The bioavailability of CdA was slightly, but not significantly higher during fasting compared to after food intake. In the fasting condition, CdA is absorbed faster, which might result in more drug escaping first-pass loss due to the saturation of liver/gut wall enzymes caused by the high portal concentration during the absorption phase. Bioavailability might also be decreased by the instability of CdA during passage through the acid environment of the stomach. However, there seems to be no major difference between fasting with and without concomitant administration of omeprazole. This suggests that acid hydrolysis in

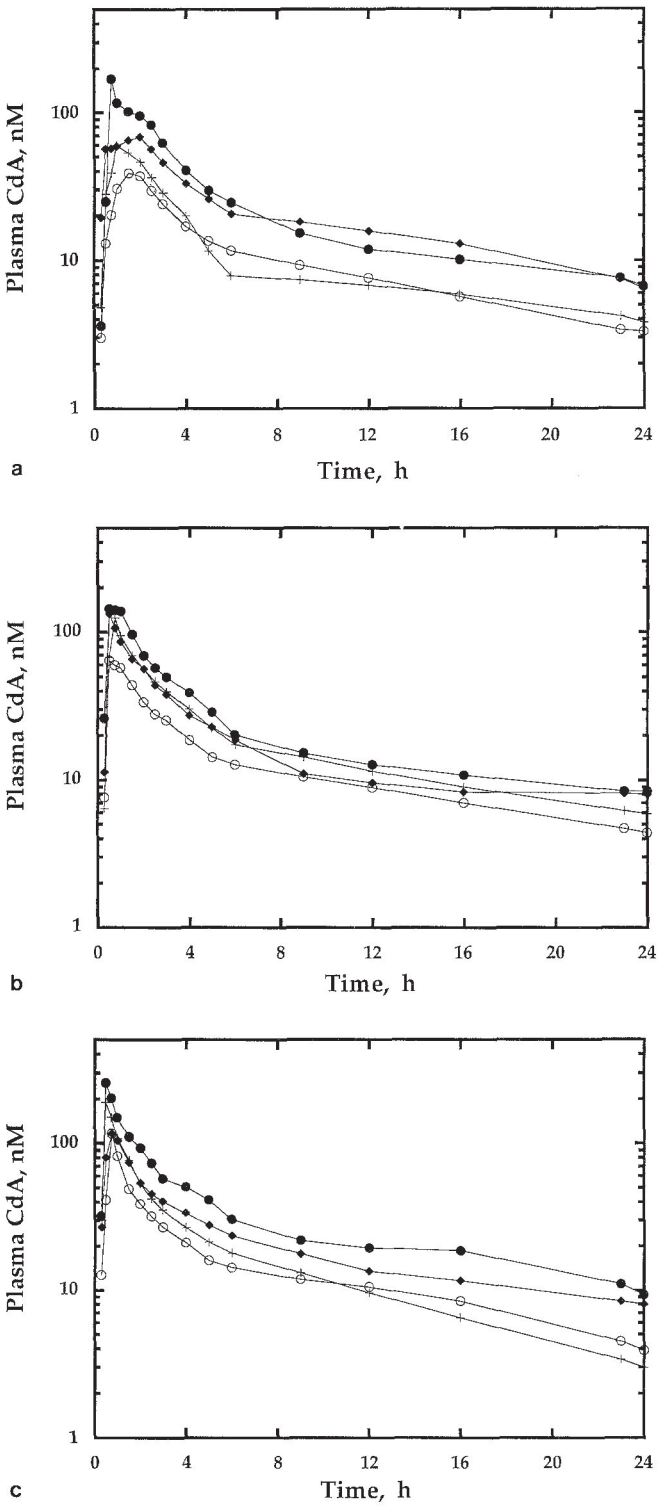

Fig. 2 a–c. Individual plasma concentration-time profiles CdA in 4 patients after administration of a single dose CdA oral (0.24 mg/kg). **a** Not fasting; **b** Fasting; **c** Fasting with concomitant

the stomach does not have a major effect on the bioavailability of CdA.

The order in which patients were given CdA after fasting or after food intake was altered, but omeprazole was given only on Day 3 due to concern that omeprazole might influence drug uptake on subsequent days if it had been given earlier. As described in Materials and Methods, the residual area was subtracted from the AUC of subsequent days, thus minimising the risk of a systematic error in estimation of bioavailability after the three modes of oral administration.

In conclusion, food slightly but not significantly reduced the bioavailability of CdA. In the fasting condition, due to quicker gastric emptying, a shorter t_{max} and a higher C_{max} were seen. Interindividual variation in bioavailability was equal after all three modes of administration. It is recommended that CdA is administered orally after an overnight fast.

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References

1. Kazimierczuk Z, Vilpo JA, Seela F (1993) Base-modified nucleosides related to 2-chloro-2′-deoxyadenosine. Helv Chim Acta (in press)
2. Plunkett, W, Saunders PP (1991) Metabolism and action of purine nucleoside analogs. Pharmacol Ther 49: 239–268
3. Seto S, Carrera CJ, Kubota M, Wasson DB, Carson DA (1985) Mechanism of deoxyadenosine and 2-chlorodeoxyadenosine toxicity to nondividing human lymphocytes. J Clin Invest 75: 377–383
4. Piro LD, Carrera CJ, Carson DA, Beutler E (1990) Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. New Engl J Med 322: 1117–1121
5. Saven A, Carrera CJ, Beutler E, Carson E, Prio LD (1991) 2-chlorodeoxyadenosine treatment of refractory chronic lymphocytic leukemia. Leukemia Lymphoma 5 [Suppl]: 133–138
6. Juliusson G, Elmhorn-Rosenborg A, Liliemark J (1992) Complete response to 2-chloro-deoxyadenosine (CdA) in B-cell chronic lymphocytic leukemia resistant to fludarabine. New Engl J Med 327: 1056–1061
7. Kay AC, Saven A, Carrera CJ, Carson DA, Thurston D, Beutler E, Piro LD (1992) 2-chloro-deoxyadenosine treatment of low-grade lymphomas. J Clin Oncol 10: 371–377
8. Santana VM, Mirro J Jr, Harwood FC, Kearns C, Schell MJ, Crom W, Blakley RL (1992) 2-chloro-deoxyadenosine produces a high rate of complete hematologic remission in relapsed acute myeloid leukemia. J Clin Oncol 10: 363–370
9. Liliemark J, Juliusson G (1991) On the pharmacokinetics of 2-chloro-2′-deoxyadenosine in humans. Cancer Res 51: 5570–5572
10. Liliemark J, Albertioni F, Hassan M, Juliusson G (1992) On the bioavailability of oral and subcutaneous 2-chloro-2′-deoxyadenosine in humans; Alternative routes af administration. J Clin Oncol, 10: 1514–1518
11. Carson DA, Wasson DB, Esparza LM, Carrera CJ, Kipps TJ, Cottam HB (1992) Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2′-arabino-fluoro-2′-deoxyadenosine. Proc Nat Acad Sci USA 89: 2970–2974
12. Kazimierczuk Z, Cottam HB, Ravankar GR et al. (1984) Synthesis of 2′-deoxytubercidin, 2′-deoxyadenosine and related 2′-deoxynucleosides via a novel direct stereospecific serum salt glycosylation procedure. J Am Chem Soc 106: 6379–6382
13. Beutler E (1992) Cladribine(2′-Chlorodeoxyadenosine). Lancet 340: 952–956
14. Liliemark J, Pettersson B, Juliusson G (1991) Determination of 2-chloro-2′-deoxyadenosine in human plasma. Biomedical Chromatogr 5: 262–264
15. Howeden CW, Forrest JAH, Ried JL (1984) Effect of single repeated doses of omeprazole on gastric acid and pepsin secretion in man. Gut 24: 707–710

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