Cellular Pharmacokinetics of 2-Chloro-2'-Deoxyadenosine Nucleotides: Comparison of Intermittent and Continuous Intravenous Infusion and Subcutaneous and Oral Administration in Leukemia Patients

Jan Liliemark and Gunnar Julissusson

Departments of Clinical Pharmacology and Oncology, Karolinska Hospital, S-104 01 Stockholm [J. L.], and Division of Clinical Hematology and Oncology, Department of Medicine, Huddinge Hospital, Huddinge [G. J.], Sweden

ABSTRACT

2-Chloro-2'-deoxyadenosine (CdA) is a new purine nucleoside analogue with major activity in lymphoproliferative diseases. Its intracellular nucleotides, in particular the 5'-triphosphate, are thought to be the pharmacologically active metabolites. The present study was undertaken to elucidate the cellular pharmacokinetics of these active metabolites in leukemia patients during CdA treatment. The concentrations of CdA in plasma and of CdA nucleotides (CdAN) in leukemic cells were measured by liquid chromatography in 69 patients with chronic lymphocytic, acute myeloid, and hairy cell leukemia after intermittent and continuous i.v. infusion, s.c. injection, and p.o. administration. The t1/2 of CdAN during the first dose interval was 13.8 h (n = 67), while after the last dose the t1/2 was 32.7 h (n = 8). The area under the concentration versus time curve was similar after intermittent and continuous infusion, 268.3 and 237.8 μM/h, respectively (n = 7). The area under the concentration versus time curve for p.o. administration (0.24 mg/kg) was slightly lower than that after intermittent infusion (0.12 mg/kg), 120.6 versus 188.8 μM/h (P < 0.05, n = 7). However, when all p.o. administrations (n = 16) were compared with all 2-h infusions in other patients with chronic lymphocytic leukemia (n = 32), there was no significant difference (149.6 versus 168.6 μM/h). The cellular concentration of CdAN was 320 times higher than the plasma concentration of CdA, but there was no correlation in individual patients (r² = 0.02, n = 69). The t1/2 of CdAN was significantly shorter in patients with acute leukemias (9 h) compared to those with chronic lymphocytic (12.9 h) and hairy cell leukemias (15.1 h). The area under the concentration versus time curve of CdAN in leukemic cells from the 11 patients with hairy cell leukemia given CdA s.c. was in the same range (179.8 μM/h) as in patients with chronic lymphocytic leukemia. The retention of CdAN in leukemic cells supports intermittent administration. The lack of correlation between cellular and plasma drug concentrations indicates that plasma drug concentrations are not useful for individualization of dose.

INTRODUCTION

CdA is a new purine nucleoside analogue which has recently emerged as the drug of choice for HCL (1-3). The results of treatment of other lymphoproliferative diseases (4-6) as well as of AML (7) are also very promising, making it one of the most interesting new anticancer drugs developed during the last decade. CdA is resistant to deamination by adenosine deaminase due to protonization at N-7 (8). Intracellularly CdA is phosphorylated by deoxycytidine kinase to its 5'-monophosphate. Its nucleotides (CdAN), presumably the 5'-triphosphate, are thought to be the active metabolites by inhibiting DNA polymerase β (9) and possibly DNA repair, causing DNA single-strand breaks. The DNA damage activates poly(ADP-ribosylation) which consumes NAD. The depletion of NAD and ATP is shown to be important for the toxic effects of CdA in vitro (10). An alternative mechanism of action is mediated through DNA fragmentation and apoptosis (11). Early studies on the pharmacokinetics of CdA revealed a rapid distribution of CdA from plasma and undetectable levels 2 h after administration (12). On the basis of these studies, continuous infusion was used as the mode of administration of choice. Later it was shown that there is a prolonged elimination phase (13, 14) and that the AUC of plasma CdA is similar after intermittent and continuous infusion (13). The metabolism of CdA in tumor cells has been studied in vitro. The retention of the CdA nucleotides was short in these studies based on wash-out experiments (15, 16). The present study was undertaken in order to describe the cellular pharmacokinetics of its active metabolite, the CdAN, in leukemic cells in patients in vivo.

PATIENTS AND METHODS

Patients and Treatment. CdA was produced at the Foundation for Diagnostics and Therapy (Warsaw, Poland) by Dr. Zygmunt Kazimierczuk (17). The doses and concentrations stated in this study where calculated using the extinction coefficient 15 AU/mm (18, 19). Sixty-nine leukemia patients were...
Cellular CdA Pharmacokinetics

Table I Patients and treatments

<table>
<thead>
<tr>
<th></th>
<th>2-h i.v. infusion</th>
<th>Continuous infusion*</th>
<th>s.c. injection*</th>
<th>p.o.*</th>
<th>2 h' + p.o.*</th>
<th>2 h' + continuous infusion*</th>
<th>2 h' + continuous infusion* + p.o.*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL</td>
<td>24</td>
<td></td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td>HCL</td>
<td></td>
<td></td>
<td>2</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>AML</td>
<td>8</td>
<td></td>
<td>2</td>
<td>11</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td></td>
<td>2</td>
<td>11</td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>69</td>
</tr>
</tbody>
</table>

*a 0.6 mg/kg/course (7 days).
*b 1.2 mg/kg/course (5 days).
*c 0.6 mg/kg/course (5 days).
*d 1.1 mg/kg/course (5 days).

The concentration of CdAN in leukemic cells was determined as follows: Leukemic cells were isolated on Lymphoprep and washed twice with PBS (8.1 g NaCl, 0.22 g KCl, and 1.14 g Na2HPO4/liter H2O at pH 7.4). The total cell volume in each sample was calculated using the cell number and median cell volume as determined on a Coulter Multisizer (Coulter Electronics, Luton, United Kingdom). The nucleotides were extracted twice with 2.5 ml 60% methanol in H2O. After evaporation, the cell extracts were reconstituted in 1 ml Tris buffer (40 mM Tris, 40 mM NaCl, 40 mM MgCl2, 40 µg/ml BSA, pH 7.5) and incubated with 5 units of alkaline phosphatase (Grade II; Boehringer Mannheim, Mannheim, Germany) for 2 h at 37°C. With this procedure >95% of endogenous 5’-triphosphate nucleotides are degraded while >95% of added CdA is recovered. The CdA was extracted with ethylacetate and the amount of CdA was determined with HPLC as for plasma concentrations. The intracellular concentration of CdAN was calculated by dividing the the amount of CdA in the alkaline phosphatase-treated extracts with the total cell volume of the samples.

Pharmacokinetic Calculations. The AUC of plasma CdA was calculated according to the trapezoid rule. The AUC and t1/2 of cellular CdAN was calculated using logarithmic regression assuming a monophasic elimination from the peak concentration. The AUC of CdAN from the start of infusion to the peak was calculated using the trapezoid rule. The t1/2 of CdA in plasma was calculated with logarithmic regression using determinations from the last three time points. To enable a comparison to other patients, the AUC of CdA and CdAN in HCL and AML patients was normalized for dose by assuming a linear relationship between dose, plasma AUC, and AUC for CdAN in leukemic cells in individual patients within the dose interval 0.085–0.22 mg/kg.

Statistical Calculations. Student’s t test for paired samples was used to assess the difference between the AUC of CdAN after i.v. and p.o. administration in nine patients where both routes of administration were used. Student’s t test for unpaired samples was used to test the difference of the AUC of CdAN between the 16 patients treated with CdA p.o. and 32 other patients treated i.v. An ANOVA was made to assess the differences in pharmacokinetic parameters between different diagnoses. Linear and logarithmic regression analyses were used to assess the correlation between parameters for plasma CdA and intracellular CdAN pharmacokinetics. All statistical calculations were made using the StatView SE + Graphics software.

Sampling Procedure. Blood was sampled in heparinized tubes at the end of the 2-h infusion and at 1, 2, 4, 8, 20, and 22 h postinfusion. In some patients, samples were taken once or twice daily after the infusion on day 5. During continuous infusion, samples were taken daily during infusions and once or twice daily after the end of infusion. In selected patients, samples were taken every 4–6 h during the first 2 days of infusion to delineate the drug accumulation during continuous infusion. After s.c. infusion, samples were taken at 30 min and at 1, 2, 4, 8, 20, and 24 h postinjection.

Determination of Drug Concentrations. The concentration of CdA in plasma was determined with HPLC as described previously (20).

Fig. 1 The concentration of CdA in plasma (lower part of the panel) and the concentration of CdA nucleotides (CdAN) in leukemic cells (upper part of the panel) in the first 16 patients with CLL given a 2-h infusion of CdA (0.12 mg/kg).
Table 2  Pharmacokinetic parameters of CdA after intermittent (2 h) and continuous i.v. infusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>2 h AUC (μM × h)</th>
<th>2 h t_{1/2} (h)</th>
<th>2 h AUC (nM × h)</th>
<th>2 h t_{1/2} (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>268.3</td>
<td>15.3</td>
<td>589.1</td>
<td>11.3</td>
</tr>
<tr>
<td>SD</td>
<td>63.1</td>
<td>4.8</td>
<td>188.1</td>
<td>3.3</td>
</tr>
<tr>
<td>CV</td>
<td>0.24</td>
<td>0.31</td>
<td>0.32</td>
<td>0.48</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*a.c.i., continuous infusion, CV, coefficient of variation.

RESULTS

Only very low concentrations of CdA (<3% of CdAN) were found in cell extracts before treatment with alkaline phosphatase, indicating that the CdA measured after alkaline phosphatase treatment represents CdAN.

Intermittent i.v. Infusion. The pharmacokinetics of plasma CdA and cellular CdAN in the first 16 patients treated is shown in Fig. 1. There was a monophasic elimination of CdAN from leukemic cells during one dose interval in contrast to CdA in plasma which showed a biphasic elimination. The t_{1/2} of CdAN during the first dose interval after 2-h infusions, s.c. injections, or p.o. administration was 13.8 ± 10.3 h (mean ± SD; n = 67) while after the fifth day the t_{1/2} was more than twice as long (Table 2). This difference is probably due to a biphasic elimination (Fig. 2) which is not appreciated during the first 24 h after infusion.

Comparison of Cellular CdAN and Plasma CdA Pharmacokinetics. The peak and trough concentrations of CdAN in leukemic cells after a 2-h infusion, with a dose interval of 24 h (n = 38), were 10.7 ± 7.1 and 2.4 ± 1.3 μM, respectively. The corresponding values for CdA in plasma was 111.9 ± 45.7 and 7.9 ± 6.7 nm. The difference between peak and trough concentrations was therefore 30.0 ± 36.5 times for CdA in

Fig. 2 The concentration of CdA in plasma (lower part of the panel) and the concentration of CdA nucleotides (CdAN) in leukemic cells (upper part of the panel) in one patient with CLL given 0.6 mg/kg CdA as 2-h infusions (O) and 4 weeks later as continuous infusion (●) of the same dose during 5 days.
plasma but only 5.1 ± 3.4 times for CdAN in leukemic cells. The cellular concentration of CdAN was 104 ± 7 and 511 ± 453 times higher than the plasma CdA concentration at the peak and trough, respectively. When the plasma and cellular AUC was compared after 2-h intermittent infusion in 44 patients, the cellular AUC of CdAN was 318 ± 194 times higher than the plasma AUC of CdA. There was no correlation between the AUC or t_{1/2} of plasma CdA (terminal t_{1/2}) and cellular CdAN in any of the analyzed patient categories (Table 3 and Fig. 3).

During continuous infusion the steady-state concentration of CdA in plasma was 27.3 ± 13.1 nm and of CdAN in leukemic cells, 9.9 ± 4.5 μM. Thus the mean difference between plasma CdA and cellular CdAN during continuous infusion was 422 ± 199 times. The pattern of plasma and cellular pharmacokinetics during intermittent and continuous infusion in one patient is shown in Fig. 2. The AUC for CdAN was similar after intermittent 2-h and continuous i.v. infusions (Table 2).

**p.o. Administration.** The AUC of CdAN in leukemic cells after p.o. administration (0.24 mg/kg) was slightly lower than that after intermittent infusion (0.12 mg/kg; Table 4). When all p.o. administrations (n = 16) were compared with 2-h infusions in all other patients with CLL (n = 32), there was no significant difference in the AUC of CdAN (149.6 ± 101.7 versus 168.6 ± 91.2).

**CdA and CdAN Pharmacokinetics according to Diagnosis.** The AUC and t_{1/2} of plasma CdA and cellular CdAN after normalization for dose in different diagnoses are shown in Table 5. There were no statistically significant differences in plasma AUC. The t_{1/2} of plasma CdA in AML patients was, however, shorter than that in CLL patients. Both the AUC and the t_{1/2} of CdAN in patients with AML was significantly shorter than those of both CLL and HCL patients. The AUC and t_{1/2} of CdA in plasma and CdAN in leukemic cells from 11 patients with HCL were similar to those in patients with CLL.

**DISCUSSION**

CdA is one of the most important new anticancer drugs developed during the last decade. Its therapeutic potential in lymphoproliferative disorders is very promising (4–6) and it has rapidly become the drug of choice for HCL (1–3). We have previously delineated the plasma pharmacokinetics of this drug (13, 14). The intracellular metabolism has been studied previously in vitro in both cell lines and in leukemic cells from patients with acute myeloid leukemia (15). In contrast, no data on the cellular metabolism in vivo have been published except a preliminary report of the present study (21). The present study delineates the cellular pharmacokinetics of CdAN in patients with CLL, AML, and HCL during intermittent and continuous i.v. infusions as well as s.c. and p.o. administration.

The method used in this study to determine the intracellular concentration of CdAN does not separate CdA from CdAN. In contrast to the CdAN, the nucleoside CdA traverses the cell membrane quite readily and most of the intracellular CdA is probably lost during the washing procedure. We have shown here that the intracellular concentration of CdA and CdAN nucleotides is several hundred times higher than the plasma concentration of CdA. Thus, if it is assumed that the extracellular and intracellular concentration of CdA is similar, the nucleoside represents only a very small part of the total intracellular drug concentration. This assumption is supported by the very small amount of CdA found in cell extracts before treatment with alkaline phosphatase. Thus, practically all of the measured intracellular drug concentration represents CdAN. Our chromatographic method easily separates CdA and 2-chloroadenosine (22). Thus ribonucleotide metabolites of CdA are not measured as CdAN.

The intracellular CdAN concentrations measured in the present study represent a mixture of the 5'-monophosphates, 5'-diphosphates, and 5'-triphosphates. In vitro studies, using radioactively labeled drug, have shown that the major intracellular metabolite is actually the monophosphate (15, 16). Thus, it seems that the first step in the bioactivation, phosphorylation by deoxycytidine kinase, is not the rate-limiting step. The same relationship is seen with other nucleoside analogues, e.g., azidothymidine (23). The triphosphate, considered to be the active metabolite, is therefore probably only a minor part of the intracellular metabolites. This has to be taken into consideration when evaluating the present study. However, it can be assumed that the CdA 5'-monophosphate and 5'-diphosphate serve as a substrate pool for further phosphorylation to the 5'-triphosphate. Therefore, there is probably a direct relationship between the intracellular concentration of CdA 5'-triphosphate and total CdAN. The determination of the low CdA 5'-triphosphate concentrations in leukemic cells in vivo requires a very sensitive methodology which is not yet available. It is therefore important to develop more specific methods which can quantitatively extract and separate the cellular metabolites of CdA. Such development is ongoing.

---

**Table 3** Correlations between plasma and cellular pharmacokinetic parameters of CdA

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AUC</th>
<th>t_{1/2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>P</td>
</tr>
<tr>
<td>CLL (n = 48)</td>
<td>0.01</td>
<td>0.50</td>
</tr>
<tr>
<td>HCL (n = 13)</td>
<td>0.08</td>
<td>0.34</td>
</tr>
<tr>
<td>AML (n = 8)</td>
<td>0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>Total (n = 69)</td>
<td>0.02</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Fig. 3** The relationship between the AUCs of plasma CdA and intracellular CdAN in 69 patients with CLL, HCL, and AML treated with CdA 0.085–0.22 mg/kg i.v. or s.c. or 0.24 mg/kg p.o.
also with other antimetabolites (25). Three of the eight patients for every single patient. Such interindividual variability is seen in carcinomas drawn are valid for groups of patients but not necessarily in overlap between the diagnosis groups. Therefore, the conclusion both plasma and cellular pharmacokinetics, there is a great differences in the metabolism of CdA and CdAN in different diagnoses.

<table>
<thead>
<tr>
<th>Plasma CdA</th>
<th>Leukemic cell CdAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (nm × h)</td>
</tr>
<tr>
<td></td>
<td>i.v. p.o. i.v. p.o.</td>
</tr>
<tr>
<td>CLL* (n = 38)</td>
<td>572.7 ± 244.3</td>
</tr>
<tr>
<td>HCL* (n = 11)</td>
<td>610.5 ± 181.1</td>
</tr>
<tr>
<td>AML(^d) (n = 8)</td>
<td>453.2 ± 308.7</td>
</tr>
<tr>
<td></td>
<td>165.5 ± 84.8</td>
</tr>
<tr>
<td></td>
<td>179.8 ± 74.2</td>
</tr>
<tr>
<td></td>
<td>89.6 ± 56.8(^e)</td>
</tr>
</tbody>
</table>

\( ^* 2\text{-h i.v. infusion.} \\
^a P < 0.05. \\
^b 2\text{-h i.v. infusion normalized for dose (0.22 mg/kg).} \\
^c P < 0.05 toward AUC for CLL or HCL. \\
^d 2\text{-h i.v. infusion normalized for dose (0.085 mg/kg).} \\
^e P < 0.05 toward t1/2 for CLL or HCL.
also much shorter than what we show here in vivo. Furthermore, the longer \( t_{1/2} \) seen when a prolonged sampling was made after the end of a treatment course indicates that there might be a biphasic elimination and an even slower elimination phase of CdAN from leukemic cells in vivo.

The lack of correlation between plasma CdA and cellular CdAN concentrations is not surprising. The intracellular CdAN concentration is a product of the plasma CdA concentration and the activity of intracellular metabolizing enzymes, both phosphorylating and dephosphorylating. The activity of these enzymes is highly variable among patients (27, 28). However, it remains to be shown whether intracellular concentrations of CdA metabolites are important for the clinical effects of CdA.

ACKNOWLEDGMENTS

We thank Birgitta Pettersson for her skillful and reliable technical assistance throughout this study and the staff at the Department of Medicine at Huddinge Hospital for the blood sampling.

REFERENCES


Cellular pharmacokinetics of 2-chloro-2'-deoxyadenosine nucleotides: comparison of intermittent and continuous intravenous infusion and subcutaneous and oral administration in leukemia patients

J Liliemark and G Juliusson


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/1/4/385

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.