PHARMACOKINETICS OF CLADRIBINE IN A RAT MODEL FOLLOWING SUBCUTANEOUS AND INTRA-ARTERIAL INJECTIONS[#]

Pollen K.F. Yeung*, Brian King, Soulatchana Narayanan and Mary Le Min Li

Pharmacokinetics and Metabolism Laboratory, College of Pharmacy and Department of Medicine, Faculties of Health Professions and Medicine, Dalhousie University, Halifax, Nova Scotia, Canada

SUMMARY

Male Sprague Dawley rats (n = 6-8 per group) weighing from 300-450 g were used for the study. Each rat received a single dose of cladribine (CdA) by ia (1 mg/kg) or sc (2 mg/kg) injection. Pharmacokinetic data were calculated by standard procedures assuming a 2-compartment open model following iv bolus using WinNonLin® and Rstrips®, and. differences between the two modes of injections were considered significance when $p < 0.05$. The results showed that plasma concentrations of CdA decreased rapidly following a biphasic decline after both ia and sc administrations. The AUC and $t_{½}$ β after a single 1 mg/kg ia and 2 mg/kg sc injection of CdA were 0.66 ± 0.34 vs 1.2 ± 0.3 μg·h/ml and 3.5 ± 2.1 vs 4.5 ± 2.2 h, respectively ($p > 0.05$). The mean absolute bioavailability following the sc injection was close to 90%. The inter-subject variability of plasma concentrations of CdA was 35% and 150% following sc and ia injections, respectively. It is

[#] This study was presented in part at the 10th Annual International Symposium on Pharmaceutical Sciences, Montreal, Quebec, Canada, May 30-June 2, 2007.

* Author for correspondence:
Pollen K.F. Yeung, Ph.D.
Pharmacokinetics & Metabolism Laboratory
College of Pharmacy
5968 College Street, Burbidge Building
Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5
e-mail: Pollen.Yeung@Dal.Ca

concluded that the rat is a reasonably good animal model to study the pharmacokinetics of CdA in plasma, and that sc injection may produce more favourable pharmacokinetic profiles than ia injection following a single dose.

KEY WORDS

cladribine, pharmacokinetics, HPLC, cancer, rats

INTRODUCTION

Cladribine (2-chlorodeoxyadenosine [CdA]) has been shown to be highly effective for treatment of several haematological malignancies, including hairy cell leukaemia and chronic lymphocytic leukaemia (CLL) /1/, and could have considerable potential also for solid tumors and autoimmune diseases /2/. CdA is a deoxypurine nucleoside analogue that resists deamination by adenosine deaminase /1/. It is phosphorylated intracellularly by deoxycytidine kinase to 2-chloro-deoxyadenosine-5′-triphosphate, which gets incorporated into DNA, and inhibits cancer progression and subsequently leads to cell death /3/. CdA is currently administered by intravenous (iv) infusion over a 7-day cycle (either continuous or 2 h/day) /4/. This mode of drug administration requires specialized staff and is inconvenient for the patient. In addition, many adverse experiences (pain, thrombosis, phlebitis) are often directly related to the iv injection method /5/. Subcutaneous injection of CdA has been shown to have similar efficacy as iv infusion for treatment of hairy cell leukaemia /6/. Recently, oral administration of CdA was being investigated for its effectiveness for treatment of relapsing-remitting multiple sclerosis (MS) although the results were not conclusive /2/.

Clinically, depending on the sampling times, the pharmacokinetics of CdA is best described by a multi-compartment open model following iv infusion /7/, with a terminal half-life ($t_{½}$ β) of about 10 hours /8/. The oral bioavailability (F) of cladribine was shown to range from 37-51% /8/. When administered subcutaneously (sc), the bio-availability of CdA is close to 100%, with peak plasma concentrations (C_{max}) occurring at approximately 30 minutes after dosing /8,9/. The bioavailability of CdA following rectal administration was only 20%,

which was attributed to degradation by bacterial enzymes /10/. Thus information about the pharmacokinetics of CdA in plasma could help to optimise dosage and the route of administration for cancer chemotherapy.

We have recently reported that the rat is a reasonably good animal model for humans to study the pharmacokinetics of cladribine in plasma. Preliminary results have shown that after a single sc injection (2 mg/kg), the mean plasma concentrations of cladribine followed a biphasic decline with a terminal half-life ($t_{½}$ β) of 10 h /11/, which is similar to humans /8/. In order to investigate whether sc injection would be a suitable alternative to iv administration, the current study employed the rodent model to compare the pharmacokinetics of CdA following a single intra-arterial (ia) and sc injection.

MATERIALS AND METHODS

Chemicals

CdA was purchased from Calbiochem (La Jolla, CA, USA), and the internal standard 3′-azido-3′-deoxythymidine (AZT) was purchased from Sigma-Aldrich Ltd (St. Louis, MO, USA). Solid phase extraction (SPE) columns were C_{18} materials (100 mg/ml) purchased commercially (Extra-Sep®, Chromatographic Specialties Inc., Brockville, ON, Canada). Solvents were HPLC grade (BDH Chem., Halifax, NS, Canada), and all other chemicals were reagent grade (Fisher Scientific, Ottawa, ON, Canada).

High performance liquid chromatography (HPLC) assay

Details of the HPLC assay have been reported in a previous publication /11/. Briefly, the HPLC system consisted of a Shimadzu LC-9A solvent delivery system, a Beckman Model 210 switching valve injector, and a Hewlett-Packard Model 1050 variable UV-VIS spectrophotometric detector. Chromatographic separation was achieved on a 3 μm minibore 250 x 2.0 mm I.D. high speed C_{18} column (Jupiter®, Phenomenex, Torrance, CA, USA), preceded with a 4 x 4 mm C_{18} guard column (Lichrocart®, EM Merck, Darmstadt, Germany) using a mobile phase of a mixture of 0.01 M potassium phosphate buffer at pH 5.0:methanol:acetonitrile (90:5:5 v/v/v), at

room temperature with a flow rate of 0.3 ml/min and operating pressure of 110 kgf/cm^2 (1.5 kpsi). CdA and AZT were extracted from plasma by solid phase extraction using 100 mg/ml C_{18} extraction cartridges (Extra-Sep®), and they were detected and quantified at 265 nm as described previously /11/.

Pharmacokinetics study

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals (UCLA) using the Canadian Council of Animal Care (CCAC) guidelines. Male Sprague Dawley rats (350-450 g) were purchased from Charles River Laboratories (Wilmington, MA, USA), and each received a single dose of CdA, 2 mg/kg by sc (n = 6) or 1 mg/kg by ia (n = 8) injection given over a 1-min period. Blood samples (0.3 ml each) were obtained from an in-dwelling carotid artery catheter serially at 0 (before injection), 0.3, 0.5, 1, 1.5, 2, 2.5, 3 and 4 hours post dose. The plasma (>0.1 ml) was immediately separated by centrifugation (4°C, 1,720 *g*, 5 min) and then stored at -80°C until analysis. An aliquot of the plasma samples (50 µl) was used for analysis of CdA by the described HPLC /11/. All samples were analyzed within 3 months after collection.

Data analysis

Pharmacokinetic variables, including area under the curve (AUC), maximum plasma concentration (C_{max}), terminal half-life ($t_{½}$ β), mean resident time (MRT), systemic clearance (CL), apparent volume of distribution ($V_β$) and volume of distribution at steady-state (Vdss) were calculated for each rat using WinNonLin® (version 5.2, Pharsight Corp., Mountain View, CA, USA) assuming a 2-compartment open model after an iv-bolus injection. Goodness-of-fit of the data was assessed empirically based on graphical display and also the Akaike Information Criterion (AIC) /12/. Pharmacokinetic parameters calculated for each administration route were compared by 2-sample *t*-test and considered significant when $p < 0.05$ (Minitab Release 14.20, State College PA, USA).

RESULTS

Plasma concentrations of CdA were highest in the first samples collected (0.3 h) from all the rats following both ia and sc injections, and declined bi-exponentially with $t_{½}$ β of 3.5 ± 2.1 and 4.5 ± 2.2 h, respectively ($p > 0.05$). The mean plasma concentration-time profiles of CdA after sc and ia injections are shown in Figure 1. The estimated mean C_{max} was higher (4.9 ± 3.8 vs 1.1 ± 0.16 μg/ml) and MRT shorter (0.98 ± 0.57 vs 3.6 ± 1.5 h) after the ia injection. These observed differences were not statistically significant ($p > 0.05$). The mean bioavailability after dose normalization was about 90%. Plasma concentrations of CdA were considerably more variable following the ia injection (Fig. 1). The data are summarized in Table 1.

DISCUSSION

There have been very few studies on the pharmacokinetics of cladribine in humans or animal models. Similar to the results obtained from our preliminary study /11/, disposition of CdA in the rats was best described by a 2-compartment open model following either sc or ia injection (Fig. 1). Absorption of CdA was very rapid after sc injection such that C_{max} was observed in the first sampling time which was less than 20 min. Due to this rapid absorption, the plasma concentration-time profiles following sc injection could be described adequately by a two compartment open model with an instantaneous input emulated by iv bolus injection. Plasma concentrations of CdA were more sustainable after sc injection as reflected by the longer MRT and $t_{½}$. These differences could be attributed mainly to the larger $V_β$ following the sc injection (3.9 ± 1.8 vs 0.14 ± 0.13 l/kg) because the CL was very similar between the two administration routes (2.3 ± 0.83 vs 2.8 ± 0.57 l/h/kg). The reason for the larger apparent $V_β$ observed after the sc injection is unclear, although it could be related to absorption and also to a greater distribution following the sc injection, which could be dose and route dependent. Nevertheless, the differences observed in these parameters were not statistically significant ($p > 0.05$) (Table 1).

The $t_{½}$s (both α and β) of CdA in rats reported here were comparable and similar to those found in humans /7,9/. $t_{½}$ α has been reported to range from 0.1-0.2 h, and $t_{½}$ β from 6-20 h /7/. These

TABLE 1

Pharmacokinetics of cladribine following a single dose in rats

	1 mg/kg ia (n = 8)	**2 mg/kg sc** (n = 6)	**Significance**[b]
AUC (μg-h/ml)	0.65 ± 0.34[a]	1.2 ± 0.24	p >0.05
C_{max} (μg/ml)	4.9 ± 3.8	1.1 ± 0.16	p >0.05
$t_{½}$ α (h)	0.35 ± 0.10	0.45 ± 0.12	p >0.05
$t_{½}$ β (h)	3.5 ± 2.1	4.5 ± 2.2	p >0.05
CL (l/h/kg)	2.8 ± 0.57	2.3 ± 0.83	p >0.05
MRT (h)	0.98 ± 0.57	3.6 ± 1.5	p >0.05
$V_β$ (l/kg)	0.14 ± 0.13	3.9 ± 1.8	p >0.05
Vdss (l/kg)	3.9 ± 2.6	5.3 ± 1.6	p >0.05

[a] Each value represents mean ± SEM.
[b] For dose normalized data as appropriate.
For abbreviations, see Methods section

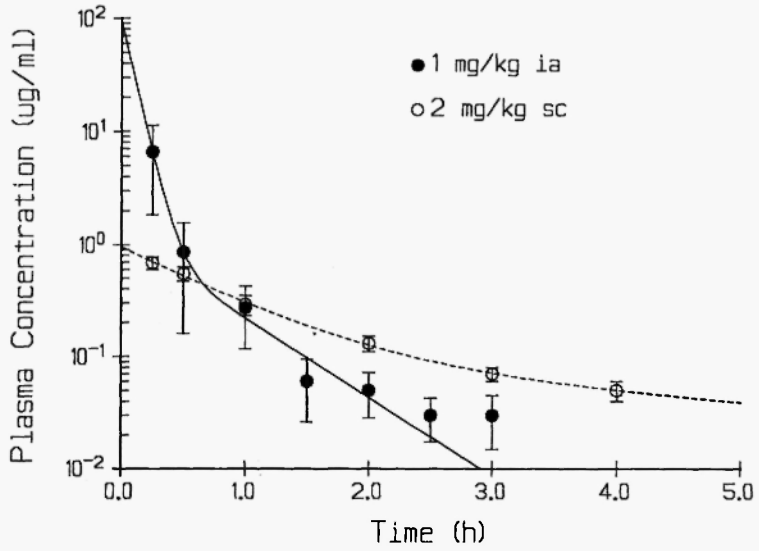

Fig. 1: Plasma concentrations of cladribine in rats after a single dose given ia or sc.

values are similar to the 0.4 and 4 h observed in the current study in rats (Table 1). The apparent volume of distribution (V_β) of CdA was reported to be about 10 l/kg /13/, and CL about 1 l/h/kg /7/. These values were also comparable to the values reported in the current study after the sc injection (Table 1). It has been reported that clinically the bioavailability of CdA was close to 100%, with t_{max} of 30 minutes after sc injection /8/. The bioavailability of 90% found in the current study also supports complete absorption with minimal degradation following sc injection in rats. The longer t_{max} observed in these earlier clinical studies could be related to the much lower dose (x 10) employed compared to the current study in rats. Furthermore, it is also very interesting to note that the inter-subject variability as shown in this study was much greater after the ia injection (AUC >50% vs 20%). A similar observation was also found in a clinical study reported previously /8/. While there was some evidence to suggest that sc cladribine is as efficacious as iv administration for treatment of hairy cell leukaemia /6/, it is still routinely administered by iv infusion. To our knowledge, this is first pharmacokinetic study reported in rats comparing bioavailability and disposition pharmacokinetics of cladribine following sc and ia administration.

In conclusion, the rat appears to be a reasonably good animal model to study the pharmacokinetics of cladribine, and sc administration may be more reliable to achieve more sustainable and less variable plasma concentrations than iv injection. This route of administration should be investigated further for clinical application.

ACKNOWLEDGEMENT

This research project was supported in part by a Nova Scotia Health Research Foundation Innovation Grant and a CIHR/Rx&D Pharmacy Summer Research Scholarship to Brian King.

REFERENCES

1. Greyz N, Saven A. Cladribine: from the bench to the bedside–focus on hairy cell leukemia. Expert Rev Anticancer Ther 2004; 4: 745-757.
2. Brousil JA, Roberts RJ, Schlein AL. Cladribine: an investigational immunomodulatory agent for multiple sclerosis. Ann Pharmacother 2006; 40: 1814-1821

3. Beutler E. Cladribine (2-chlorodeoxyadenosine). Lancet 1992; 340: 952-956.
4. Robak T, Wierzbowska A, Robak E. Recent clinical trials of cladribine in hematological malignancies and autoimmune disorders. Rev Rec Clin Trials 2006; 1: 15-34.
5. von Rohr A, Schmitz SF, Tichelli A, et al. Treatment of hairy cell leukemia with cladribine (2-chlorodeoxyadenosine) by subcutaneous bolus injection: a phase II study. Ann Oncol 2002; 13: 1641-1649.
6. Juliusson G, Heldal D, Hippe E, et al. Subcutaneous injections of 2-chlorodeoxyadenosine for symptomatic hairy cell leukemia. J Clin Oncol 1995; 13: 989-995.
7. Johnson SA. Clinical pharmacokinetics of nucleoside analogues: focus on haematological malignancies. Clin Pharmacokinet 2000; 39: 5-26.
8. Liliemark J, Albertioni F, Hassan M, Juliusson G. On the bioavailability of oral and subcutaneous 2-chloro-2′-deoxyadenosine in humans: alternative routes of administration. J Clin Oncol 1992; 10: 1514-1518.
9. Liliemark J. The clinical pharmacokinetics of cladribine. Clin Pharmacokinet 1997; 32: 120-131.
10. Liliemark J, Albertioni F, Edlund C, Juliusson G. Bioavailability and bacterial degradation of rectally administered 2-chloro-2′-deoxyadenosine. J Pharm Biomed Anal 1995; 13: 661-665.
11. Yeung PK, Ferguson C, Jarrar A, King B, Li ML. Development and validation of a sensitive and specific HPLC assay of cladribine for pharmacokinetics studies in rats. J Pharm Pharm Sci 2007; 10: 229-234.
12. Gabrielsson J, Weiner D. Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications. Stockholm: Swedish Pharmaceutical Press, 2000.
13. Liliemark J, Juliusson G. On the pharmacokinetics of 2-chloro-2′-deoxyadenosine in humans. Cancer Res 1991; 51: 5570-5572.