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Original Communication

# Hemodynamic effects of cladribine in a restrained rat model

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## ABSTRACT

Hemodynamic effect of cladribine (CdA) was studied using a rat model after single dose. Male Sprague Dawley (SD) rats weighing between 300 - 400 g (n = 8 - 12 per group) were used. Each rat was acclimatized for one week before experiment, and then received a single dose of either 1 mg/kg CdA by intra-arterial injection (ia) or 2 mg/kg of the drug subcutaneously (sc). Hemodynamic recordings were collected via an indwelling catheter implanted into a left carotid artery continuously up to 3 hours after drug administration. A control group received saline and the same treatment as described for the CdA group. Differences between the treatment groups and control were assessed by ANOVA and *paired student's t-test*, and considered significance when $p<0.05$. Systolic and diastolic blood pressure (SBP and DBP) were significantly increased shortly after CdA injection and continued to increase for 3 hours for the duration of the experiment ($p<0.05$). By the end of the experiment, SBP was increased significantly from 130 ± 13 to 152 ± 17 mmHg (+17%) and DBP 106 ± 8.1 to 124 ± 16 mmHg (+17%) after the 1 mg/kg ia injection (paired t-test $p<0.05$). Blood pressure was also increased after the 2 mg/kg sc dose, but the increase did not reach statistical significance. Further, heart rate (HR) was decreased significantly from 453 ± 54 to 422 ± 53 bpm (-7%) and 449 ± 70 to 386 ± 69 bpm (-14%) following both 1 mg/kg ia and 2 mg/kg sc injections (paired t-test $p<0.05$). In conclusion, CdA increased SBP and DBP, but decreased HR following a single dose in the rat model. The hemodynamic effects were greater after ia injection.

**KEYWORDS:** hemodynamics, SBP, DBP, HR, cladribine, rat

## INTRODUCTION

Cladribine (CdA) is one of the first prototype purine nucleoside anti-cancer agents shown to be highly effective for treatment of hairy cell leukemia and acute and chronic myeloid leukemia, and has considerable potential for lymphomas and other forms of solid tumors [1]. It is also one of the orally effective agents which have shown to have great promise for treatment of multiple sclerosis [2, 3, 4]. CdA is a prodrug and must be phosphorylated intracellularly to cladribine-monophosphate (MP) by the nuclear/cystosol enzyme deoxycytidine kinase (dCK) and the mitochondrial enzyme deoxyguanosine kinase. The cytotoxicity depends mainly on accumulation of cladribine-triphosphates (TP) after phosphorylation of cladribine-MP by nucleoside kinases [5]. While the nucleoside anti-cancer drugs are highly effective against susceptible tumors, they can cause serious adverse cardiac effect [6], which can manifest in the forms of ischemia, heart failure, arrhythmias, and myocardial infarction [7]. Further, resistance to CdA may develop after prolonged usage [5]. Although the mechanisms of cardiac toxicity and

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cancer resistance are not currently known, they could be related to interference with energy metabolism within the cardiovascular system either directly or indirectly [8] and deficiency of dCK activity [5]. Further studies to the cause and how to minimize the risk of cardiac toxicity and cancer resistance associated with these nucleosides could greatly improve their efficacy and safety, and optimize their use in targeted therapy.

We have recently shown that the rat is a working model for pharmacokinetic study of CdA following parenteral administration [9]. The current study uses the rat model to assess for the first time hemodynamic effects of CdA after intra-arterial (ia) or subcutaneous (sc) injection.

## METHODS

CdA was purchased from Calbiochem (La Jolla, CA, USA). Male SD rats weighing between 300 - 400 g (n = 6 - 8 per group) were purchased from Charles River Laboratories (Wilmington, MA, USA), and they were acclimatized for one week before experiment. An indwelling catheter made of silastic® tubing (PE-50, Dow Corning Corp., MI, USA) was implanted into the left carotid artery of each animal under general anaesthesia for hemodynamic recording as described previously [10]. After recovery from the surgery (24 - 48 h), each rat received either saline (n = 16), a single dose of CdA (2 mg/kg) by sc (n = 10) or 1 mg/kg by ia (n = 8) injection given over a 1-min period. Hemodynamic recordings including systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were collected continuously up to 3 hours after drug administration using a TruWave disposable pressure transducer (Model PX601, Edwards Lifesciences Canada, Inc., Mississauga, ON, Canada) coupled to a Siemens hemodynamic monitor (Sirecust 400) and chart recorder (Siredoc) (Erlangen, FRG) as previously described [11-14]. Differences between the treatment groups and control were assessed by ANOVA and *paired student's t-test*. Dunnett's test was used for post hoc comparisons when a significant treatment effect was observed and the effects considered significance when $p<0.05$ (Minitab® Inc., Release 16, State College, PA, USA).

## RESULTS

The effects of CdA on cardiovascular hemodynamics (SBP, DBP and HR) were highly significant after injection (ANOVA $p = 0.000$) compared to control group. SBP and DBP started to increase shortly after injection and continued to increase for the duration of the experiment ($p<0.05$) (Figure 1). By the end of the experiment,

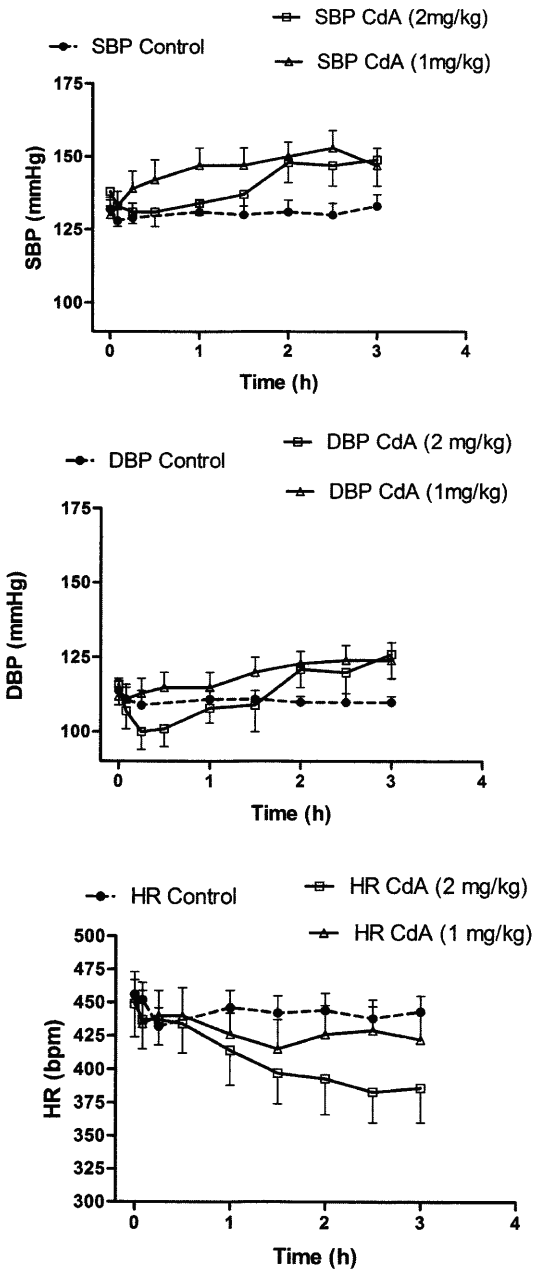


**Figure 1.** Hemodynamic effects of CdA after a single dose.

**Table 1.** Hemodynamic effects of CdA in a rodent model.

| Treatment/Hemodynamic Parameters | SBP (mmHg) | | DBP (mmHg) | | HR (bpm) | |
|---|---|---|---|---|---|---|
| | 0 hr | 3 hr | 0 hr | 3 hr | 0 hr | 3 hr |
| Control | 132 ± 16 | 133 ± 16 | 114 ± 15 | 110 ± 9 | 456 ± 42 | 443 ± 45 |
| 1 mg/kg CdA (ia) | 130 ± 13 | 152 ± 17* | 106 ± 8.1 | 124 ± 16* | 453 ± 54 | 422 ± 53* |
| 2 mg/kg CdA (sc) | 135 ± 15 | 148 ± 26 | 112 ± 18 | 126 ± 24 | 449 ± 70 | 386 ± 69* |

*p < 0.05 vs 0 hr (student's paired t-test).

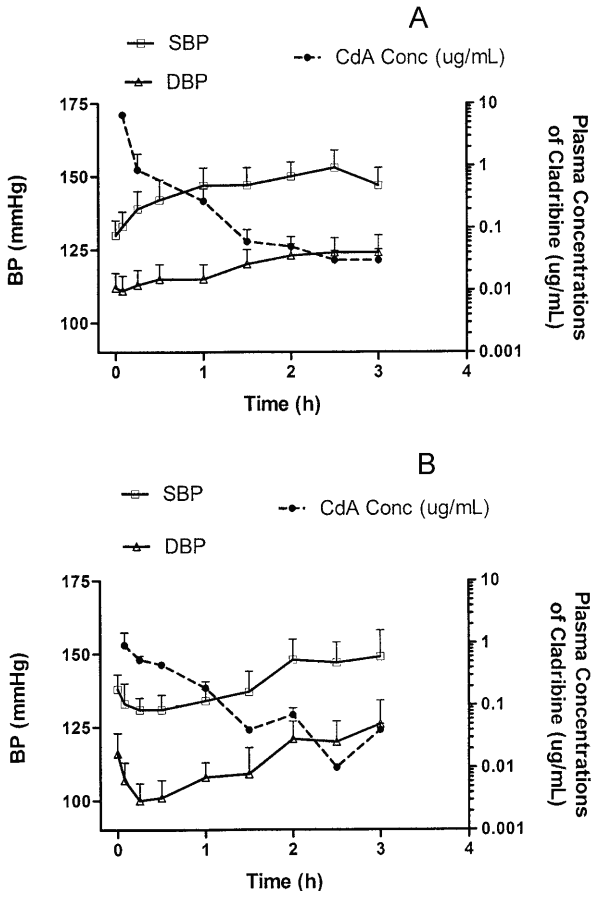


**Figure 2.** Hemodynamic effects and plasma concentration time plot after a single dose of CdA (A = 1 mg/kg ia; B = 2 mg/kg sc)

SBP was increased from 130 ± 13 to 152 ± 17 mmHg (+17%) and 135 ± 15 to 148 ± 26 mm Hg (+10%), and DBP 106 ± 8.1 to 124 ± 16 mmHg (+17%) and 112 ± 18 to 126 ± 24 mmHg (+13%) after the 1mg/kg ia and 2 mg/kg sc dose, respectively, although only the increase after the ia injection was statistically significant (*paired student's t-test* p<0.05). On the contrary, HR was decreased significantly from 453 ± 54 to 422 ± 53 bpm (-7%) and 449 ± 70 to 386 ± 69 bpm (-14%) following the 1 mg/kg and 2 mg/kg, respectively (*paired student's t-test* p<0.05). While the effect on blood pressure was greater after the ia dose, the HR effect was greater after the sc injection (Table 1).

There were no apparent correlations between plasma concentrations of CdA and hemodynamic effects. While the concentrations of CdA declined after injection, the blood pressure (SBP and DBP) remained significantly higher than baseline or pre-treatment level, and the HR continued to decline for the remainder of the experiment (Figure 2). The Pearson correlations calculated between plasma CdA concentration and the hemaodynamic variables (SBP, DBP, and HR) were not significant (p>0.05).

## DISCUSSION

Many anti-cancer drugs including the nucleosides are known to have potential for cardiovascular adverse effects [15, 7, 16]. Unlike doxorubicin which has a predictable, direct and dose dependent cardiotoxicity which occurs in > 20% of patients [15, 17, 18], cardiac adverse effects attributed to CdA is much less common. In an *in vitro* study, we did not detect any drug related cardiac cell death at concentrations 10 times higher than doxorubicin using rat myoblast (H9C2) cell line [19]. This suggests that cardiovascular adverse effects after CdA may be attributed to their effect on the vascular system.

The current study reported for the first time, significant hemodynamic effect after a single injection of CdA albeit that the dosage employed

was about 10 times the daily dosage recommended for clinical use [20]. We have shown previously in zebrafish model that 1 mg/kg of CdA given by intraperitoneal injection twice daily for 3 doses inhibited increase of ATP concentrations in RBC induced by diltiazem [21]. While the mechanism for the blood pressure increase is not known, it could be related to the effect of CdA on ATP metabolism in RBC which may affect the clotting mechanism in the blood. This is supported by the lack of a direct relationship between plasma CdA concentrations and hemodynamic response indicating a delayed response which sustained long after the injection when plasma CdA concentrations approached undetectable level (Figure 2). It is interesting to note that while CdA increased SBP and DBP, it also decreased HR. It is not clear if these effects are related to the effect on ATP metabolism in RBC or an indirect compensatory mechanism in response to the increase in blood pressure. The more rapid increase of blood pressure after the ia injection was likely attributed to the higher plasma CdA concentrations attained immediately after injection (Figure 2). However, it is not clear why the HR effect was greater after the sc injection. Further study of the effect of prolonged exposure to CdA on ATP metabolism in RBC, blood clot and cardiovascular hemodynamics is warranted.

## CONCLUSION

The study provides pilot results to indicate high dose of CdA can significantly increase SBP and DBP, but lowered HR in a restrained rat model. The hemodynamic effects may be due to an indirect mechanism affecting ATP metabolism in RBC which warrants further investigation.

## ACKNOWLEDGMENT

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