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Original Communication

Effect of doxorubicin on cardiovascular hemodynamic and RBC concentrations of ATP in rats *in vivo*

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ABSTRACT

The cardiovascular effect of doxorubicin (DOX) was investigated in a Sprague Dawley (SD) rat model *in vivo* which allowed continuous cardiovascular hemodynamic monitoring, and serial blood sample collection for measurement of RBC concentrations of ATP. The rats were randomly divided into 2 groups. Group A (n = 8) received DOX 10 mg/kg in normal saline by subcutaneous (sc) injection twice daily for 4 doses. Group B (n = 11) received the same injections with normal saline. Blood samples (0.3 mL each) were obtained from each rat before the last injection (Time 0), and at 0.05, 0.25, 1, 1.2, 1.5, 2, 3, 4, 5 and 6 hours after the injection. Hemodynamic recording was collected continuously throughout the experiment. Difference of response between groups was considered significant at $p < 0.05$ (t-test). The systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) before the last DOX dose vs control (in parenthesis) were 119 ± 7 (vs 123 ± 11 mmHg), 87 ± 12 (vs 104 ± 11 mmHg, $p < 0.05$), and 386 ± 27 (vs 378 ± 48 bpm), respectively. The blood pressure fell gradually after the last injection in both groups, and by the end of the experiment the SBP was significantly lower in the DOX treated group (85 ± 9 vs 103 ± 15 mmHg, $p < 0.05$). There was no difference in the red blood cell (RBC) concentrations of ATP between the DOX treated rats and control (1.52 ± 0.53 vs 1.69 ± 0.44 mM, $p > 0.05$). The results suggest that DOX decreased blood pressure but had little effect on HR or RBC concentrations of ATP.

KEYWORDS: doxorubicin, hemodynamics, SBP, DBP, HR, ATP, rat

INTRODUCTION

Doxorubicin (DOX) is an anthracycline widely used in chemotherapy due to its efficacy in fighting a wide range of cancers. Although highly effective against a wide variety of cancers, it has serious cardiac adverse effects and 50% of the patients developing congestive heart failure could die from the adverse event [1-3]. The mechanism of cardiac toxicity is not currently known although it could be related to oxidative stress mechanism which interferes with energy metabolism within the cardiovascular system [4-6]. Dexrazoxane is the only protective agent currently approved clinically for DOX induced cardiac injury [7, 8]. However, its effectiveness is considered limited and a more effective and clinically applicable preventive treatment still needs to be identified [3]. Further studies to the cause and how to minimize the risk of cardiac toxicity and cancer resistance associated with DOX could greatly improve their efficacy and safety, and optimize their use in targeted therapy.

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We have recently shown that the rat is a relatively good working model for pharmacokinetics and cardiovascular effect of the nucleoside anti-cancer drug cladribine (CdA) following parenteral administration [9, 10], and that red blood cell (RBC) concentrations of ATP may be useful biomarker as a measures for cardiovascular protection and toxicity. The current study uses the rat model to assess for the first time the cardiovascular hemodynamic effects of DOX and its effect on RBC concentrations of ATP after multiple doses by subcutaneous (sc) injection.

METHODS

DOX hydrochloride was purchased from Euroasian Chemical PVT Ltd. (Mumbai, India). Male Sprague Dawley (SD) rats weighing between 250-300 g with a silastic catheter implanted into a carotid artery were purchased from Charles River Laboratories (Wilmington, MA, USA). They were acclimatized for one week with free access to food and water *ad libitum* before experiment. The rats were randomly divided into 2 groups. Group A (n = 8) received DOX 10 mg/kg in normal saline (5 mg/mL) by subcutaneous (sc) injection twice daily for 4 doses. Group B (n = 11) received the same injections with normal saline (2 mL/kg). The rats were kept in a cage with free access to drinking water during experiment (Figure 1). Blood samples (0.3 mL each) were obtained from each rat from the carotid artery catheter using a swivel-tether system (Figure 1) before the last injection (Time 0), and at 0.05, 0.25, 1, 1.2, 1.5, 2, 3, 4, 5 and 6 hours after the injection. Hemodynamic recordings including systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were collected from the carotid artery catheter continuously up to 6 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 [10-14]. Concentration of ATP in the RBC was determined by a previously reported HPLC [15]. Differences between the treatment and control groups were assessed by *paired and upaired student's t-test* and the effects considered significance when $p < 0.05$ (Minitab® Inc., Release 16, State College, PA, USA).

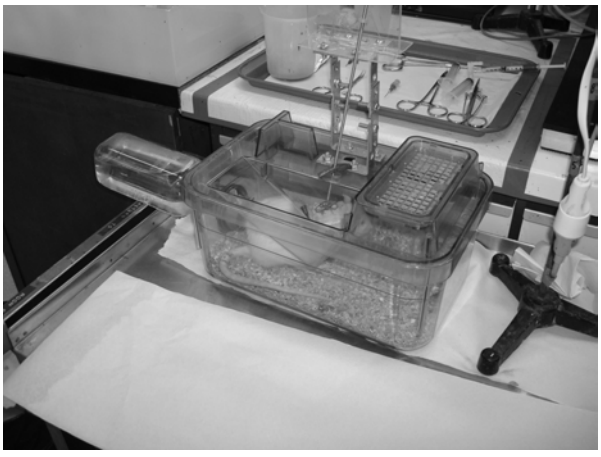

Figure 1. Experimental rat model.

RESULTS

One of the rats treated with DOX died 5 hrs after the last injection. The SBP, DBP and HR before the last DOX dose vs control (in parenthesis) were 119 ± 7 (vs 123 ± 11 mmHg), 87 ± 12 (vs 104 ± 11 mmHg, $p < 0.05$), and 386 ± 27 (vs 378 ± 48 bpm), respectively. The blood pressure (SBP and DBP) fell gradually after the last injection, although only the decrease of SBP was statistically significant in both group ($p < 0.05$ pair t test) (Table 1). By the end of the experiment the SBP, DBP, and HR were 85 ± 9 (vs 103 ± 15 mmHg, $p < 0.05$), 76 ± 9 (vs 82 ± 16 mmHg) and 370 ± 58 (vs 355 ± 39 bpm) (Table 1 and Figure 2). The RBC concentrations of ATP before the last DOX injection were 1.33 ± 0.38 mM (vs 1.41 ± 0.32 mM), which increased gradually throughout the experiment in both DOX treated and control rats ($p < 0.05$ pair t-test) (Figure 2). By the end of experiment, the RBC ATP concentration in the DOX treated rats were 1.79 ± 0.55 mM (vs 2.02 ± 0.67 mM) (Table 1). There were significant correlations between RBC concentrations of ATP and cardiovascular hemodynamic parameters (SBP, DBP, and HR) in the control rats, but only the correlations with blood pressure (SBP and DBP) were significant in the DOX treatment group (Figure 3).

Table 1. Effect of DOX on cardiovascular hemodynamics and RBC concentrations of ATP.

	DOX (10 mg/kg)	Normal Saline
SBP -T0 (mmHg)	119 ± 7	123 ± 11
SBP - Tlast (mmHg)	85 ± 9*,**	103 ± 15**
DBP - T0 (mmHg)	87 ± 11*	104 ± 11
DBP - Tlast (mmHg)	76 ± 9	82 ± 16**
HR - T0 (bpm)	386 ± 27	391 ± 26
HR - Tlast (bpm)	370 ± 58	355 ± 39
[ATP] in RBC - T0 (mM)	1.33 ± 0.38	1.41 ± 0.32
[ATP] in RBC - Tlast (mM)	1.79 ± 0.55**	2.02 ± 0.67**

*p < 0.05 vs control (*t test*)
**p < 0.05 vs T0 (*paired t-test*)

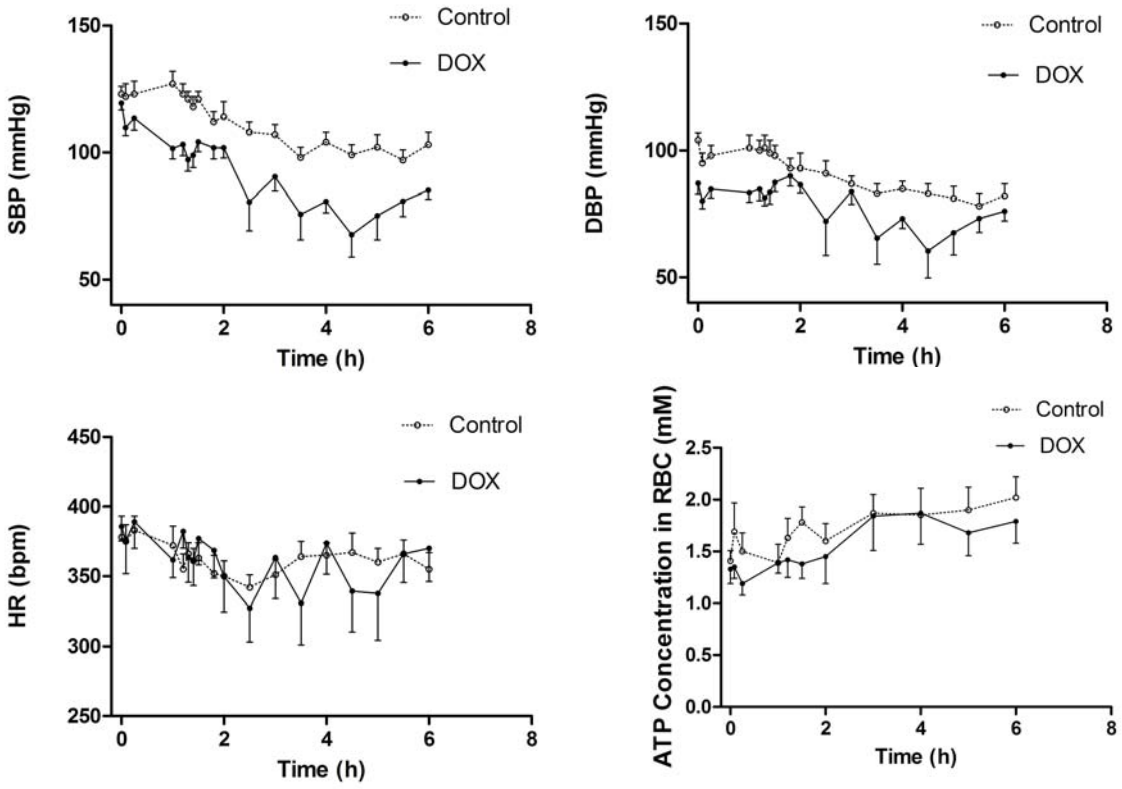

Figure 2. Effect of DOX on cardiovascular hemodynamics and RBC concentrations of ATP.

DISCUSSION

The anthracycline anti-cancer drugs such as DOX are known to have cardiovascular adverse effect [16-18] which is predictable, direct and dose dependent cardiotoxicity and can occur in > 20% of patients [2, 16, 19]. In an *in vitro* study, significant cardiotoxic effect of DOX was observed at uM range using rat myoblast (H9C2) cell line [20]. Despite extensive research to determine the mechanism of the cardiotoxicity, it is still a very

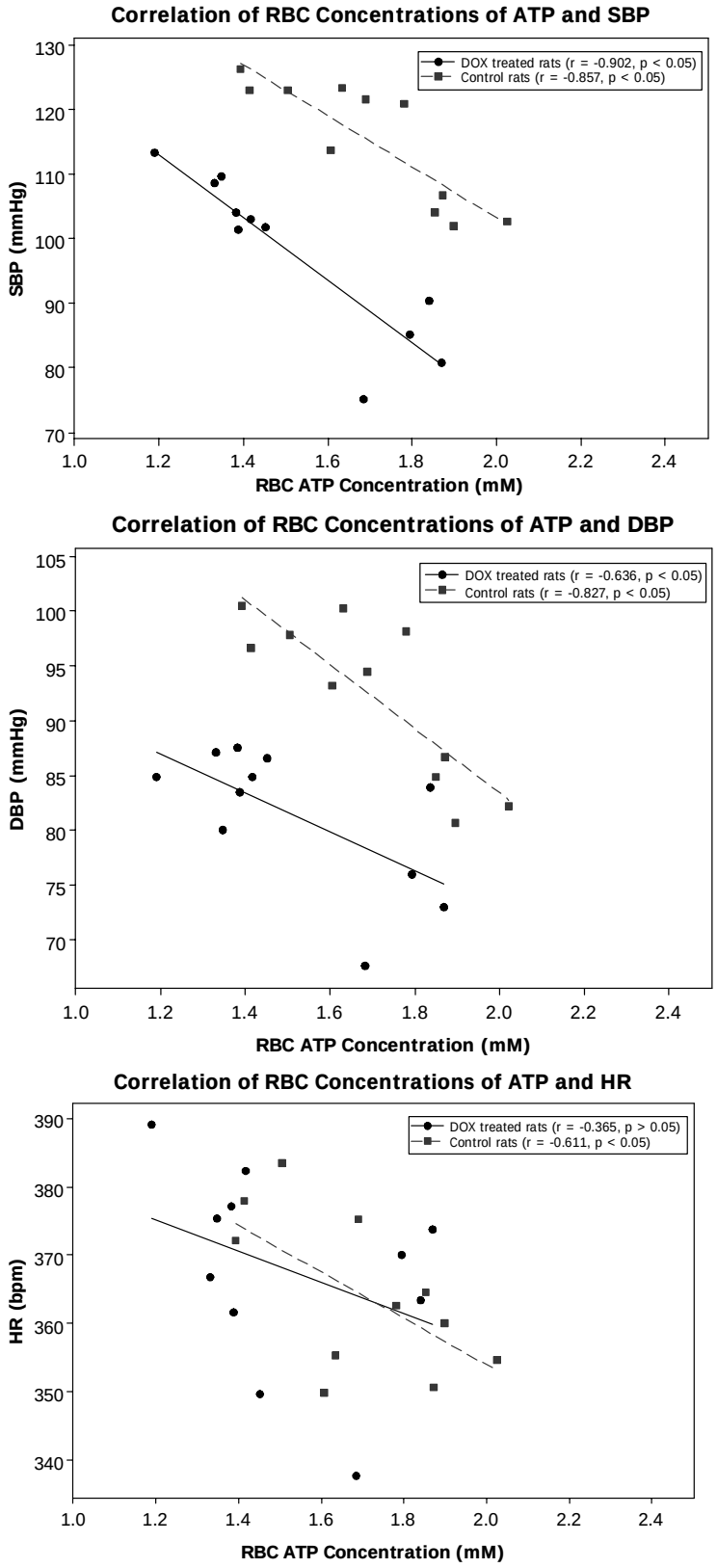

Figure 3. Correlations of RBC concentrations of ATP and cardiovascular hemodynamic.

controversial topic [21]. It is believed that an oxidative stress mechanism generating reactive oxygen species (ROS) which cause cardiac damage and altered energy metabolism in the cardiovascular system and mitochondrial function [6, 22, 23]. This could lead to myocardial ischemia or infraction and subsequently congestive heart failure [5]. Thus anti-oxidants and agents which improve oxygen supply and demand balance within the cardiovascular system can reduce cardiotoxicity induced by DOX *in vivo* [24-28] and *in vitro* using cell culture model [20].

Previous studies have shown low dose of DOX (1.5 mg/kg/week for several weeks) induced signs of cardiotoxicity [25, 29], and acute toxicities could be demonstrated with a single dose of 10 mg/kg or higher [30-32]. The toxicities are manifested in the experimental models by changes in serum and tissue biochemistry (e.g. increase serum creatine kinase), hemodynamic and electroncardiographic changes signaling cardiac dysfunctions (e.g. QTc prolongation), decrease heart-to-body ratio, and morphology changes in cardiac tissues (e.g. myofibrillar disarrangement) [32]. An altered expression of cytochrome P-450 and arachidonic acid metabolism and perhaps other cardiac functions as well have been reported [30].

The current study reported a significant blood pressure lowering effect after 10 mg/kg DOX given twice daily for 4 doses by subcutaneous injection which is about 10 times the recommended clinical dosage for DOX (30 mg/m^2/day [33]. The results are consistent with an early report which also showed a decrease in blood pressure and heart rate after a single 20 mg/kg dose of DOX [23]. On the contrary, smaller doses of DOX (2 mg/kg) given daily over a week (cumulatively dosage of about 15 mg/kg) was shown to increase SBP, but had no effect on HR [34]. The difference of the hemodynamic effect of DOX observed from these studies is likely attributed to the DOX dosage and how it was administered. It is known that cardiotoxicity induced by DOX is progressive, and that a single larger dose can cause more damage and greater mortality than multiple smaller doses [29]. The mechanism for the hemodynamic changes could be attributed to cardiac damage by DOX, which can be observed in acute toxicity induced by high dose of DOX as shown in the current study.

We had previously shown that RBC concentrations of ATP may be an important indicator for post-exercise hypotension and cardiovascular protection in rats [35]. An increase of RBC concentrations of ATP was demonstrated in zebrafish model treated with the anti-ischemia drug diltiazem, and the effect was blocked by the anti-cancer drug cladribine [36]. The lack of effect of DOX on RBC concentrations of ATP as shown in the current study (Table 1 and Figure 2) suggests that acute toxicity induced by DOX does not alter circulating ATP concentrations in the RBC. However, it remains to be tested if ATP metabolism in the RBC may be affected after chronic use of DOX.

It is also very interesting to note that there was an increase of RBC concentrations of ATP towards the end of the experiment for both control and DOX treated rats ($p < 0.05$, Table 1). Highly significant correlations were obtained between RBC concentrations of ATP and the decrease in blood pressure (SBP and DBP) in both groups of rats (Figure 3). We had reported previously a significant correlation between RBC ATP concentrations and DBP only in exercise rat, but not in rats without exercise [35]. The discrepancy could be attributed to the previous experimental condition when the rats were kept in a restrainer as opposed to in a non-restrained cage environment employed in the current study (Figure 1). Similar to the results from the previous study, the correlations with HR were considerably weaker and not significant for the DOX treated rats (Figure 3). The difference may be explained by different stress level imposed on the animals under a restrained versus freely moving condition.

CONCLUSION

The study provides pilot results to show acute toxicity of DOX may be induced by twice daily dose of 10 mg/kg for two days. The toxicity included significant decrease of blood pressure, but not HR or RBC concentrations of ATP.

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