Effects of ramipril and darbepoetin on electromechanical activity of the heart in doxorubicin-induced cardiotoxicity

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Cardiac toxicity is a deadly condition caused by impaired cardiac activity similar to congestive heart failure (CHF). Doxorubicin (DOX) is a potent anticancer drug having a potential of myocardial damage [1]. Doxorubicin induced experimental toxicity has been characterized by heart failure and anaemia [2]. In human patients, risks for the use of anthracyclines have been reported associated with cardiac death which is the most prevalent of QT prolongation that triggered Torsades de Pointes [3]. The anaemia of CHF patient is evidently due to the excessive cytokine production [4]. Pre-treatment with erythropoietin hormone alone protected the myocardium against DOX induced impaired cardiac function and cardiomyocyte apoptosis [5]. Therefore, the present study was designed to investigate the effects of ramipril and darbepoetin alfa, used either alone or together, on electromechanic activity of heart in an experimental toxicity model.

All animals received humane care and the protocol has been approved by the local ethics committee (AUHADYEK/2013/67). Forty 6-month-old Sprague–Dawley male rats were divided into 5 groups. The Control group had no medication. The DOX group received 2.5 mg/kg i.v. DOX hydrochloride (Adriblastina, Pfizer Inc.) from tail veins weekly for 3w. The DOX + RAM group received 2.5 mg/kg i.v. DOX plus 1 mg/kg p.o. ramipril (Delix, Pharma Vision) via gavage daily for 4w. The DOX + DP group received 2.5 mg/kg i.v. DOX plus 10 μ/kg i.p. darbepoetin alfa (Aranesp, Amgen Inc.) weekly for 3w. The DOX + RAM + DP group had 2.5 mg/kg i.v. DOX plus the same amount and duration of ramipril and darbepoetin. Sampling and measurement were obtained at the 4th week.

The electrocardiographic (ECG) and echocardiographic findings were obtained from the conscious rats. Standard leads were recorded (MP 150, BIOPAC Inc.). The intervals were measured over the lead II aligned by the other leads (Fig. 1) using a standard measurement method [6]. Corrected QT (QTc) was adjusted for heart rate according to the Bazett's formula [$QTc = QT/\sqrt{RR}$]. The echocardiographic examination of hearts was achieved using Vivid I portable echo system (GE Healthcare Inc., Norway) with 11.5 MHz sector probe (10S-RS). Blood counts were determined using a rat specific haematology analyzer (Abacus Vet5, Diatron Inc.). Tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) were analyzed in the sera samples. The ELISA kits for rat TNF-α and rat IL-6 were used by sandwich ELISA technique (Invitrogen). Tissue samples of hearts were fixed in 10% formalin buffered solution. After the routine histopathology processing, paraffin sections were stained with Hematoxylin and Eosine. Data were compared between groups using one-way ANOVA (SPSS Inc.). A statistical significance was defined by $P < 0.05$ with multiple comparison analysis of Duncan's test.

The parameters of ECG, echocardiography, haematology and cytokines were presented in Table 1. Briefly, the QRS duration lengthened in the DOX group ($P < 0.01$). The QRS duration in DOX + RAM group was lower than DOX group ($P < 0.01$). The injection of DOX prolonged the QTc in the DOX group ($P < 0.001$). A prevention of QTc prolongation in the treatment groups (DOX + RAM, DOX + DP, DOX + RAM + DP) was observed ($P < 0.05$). Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) in DOX treated groups were lower than Control group ($P < 0.001$). Doxorubicin injection significantly decreased RBC counts and HTC levels in DOX group ($P < 0.001$) and these decreases were prevented in DOX + RAM, DOX + DP and DOX + RAM + DP groups comparing with DOX group. The mean tHb concentration was significantly higher in DOX + RAM + DP group comparing with DOX group. TNF-α and IL-1β levels were significantly higher in the DOX group ($P < 0.001$). A reduction in IL-1β level was detected in treatment groups compared to DOX group. TNF-α level decreased only in RAM treatment compared to DOX group. Severe hyperemia and some hemorrhagic foci were observed in the histopathologic sections of hearts in DOX group. There was no hyperemia and haemorrhagia in DOX + DP group and DOX + RAM + DP group.

In the present study, weekly DOX administrations induced a severe cardiotoxicity regarding the disturbances of electromechanic activity. DOX injections produced a marked decrease in LVEF and prolongation of QTc interval along with anaemia. The treatment of CHF consists of ACE inhibitors for systolic dysfunction with decreased LVEF [7]. The use of DP alpha in anaemic patients with CHF increases Hb levels [8]. Marked prolongations of QTc interval and QRS duration, measured in the DOX group, indicate conduction abnormalities for cardiomyopathy and cardiotoxicity. The prevention of the QTc prolongation by the administration of RAM and DP may be a noticeable indication of the efficacy. Surprisingly, the administration of DP alone has a similar effect as the RAM. The QRS widening has been reversed by daily RAM administration in the DOX + RAM group. Therefore, the ACE inhibitor and the erythropoietin hormone used herein have clear protective effects directly on the heart conduction system.

A significant reduction of LVEF has been observed early after anthracycline chemotherapy in human patients [9]. The marked decrease in LVEF clearly indicates the induction of LV systolic dysfunction by DOX injection. Both DP and DP + RAM administrations did not prevent the decrease in LVEF and LVFS since the DOX injections had been continued [10]. In this experiment, the rats have lower levels of erythrocyte counts

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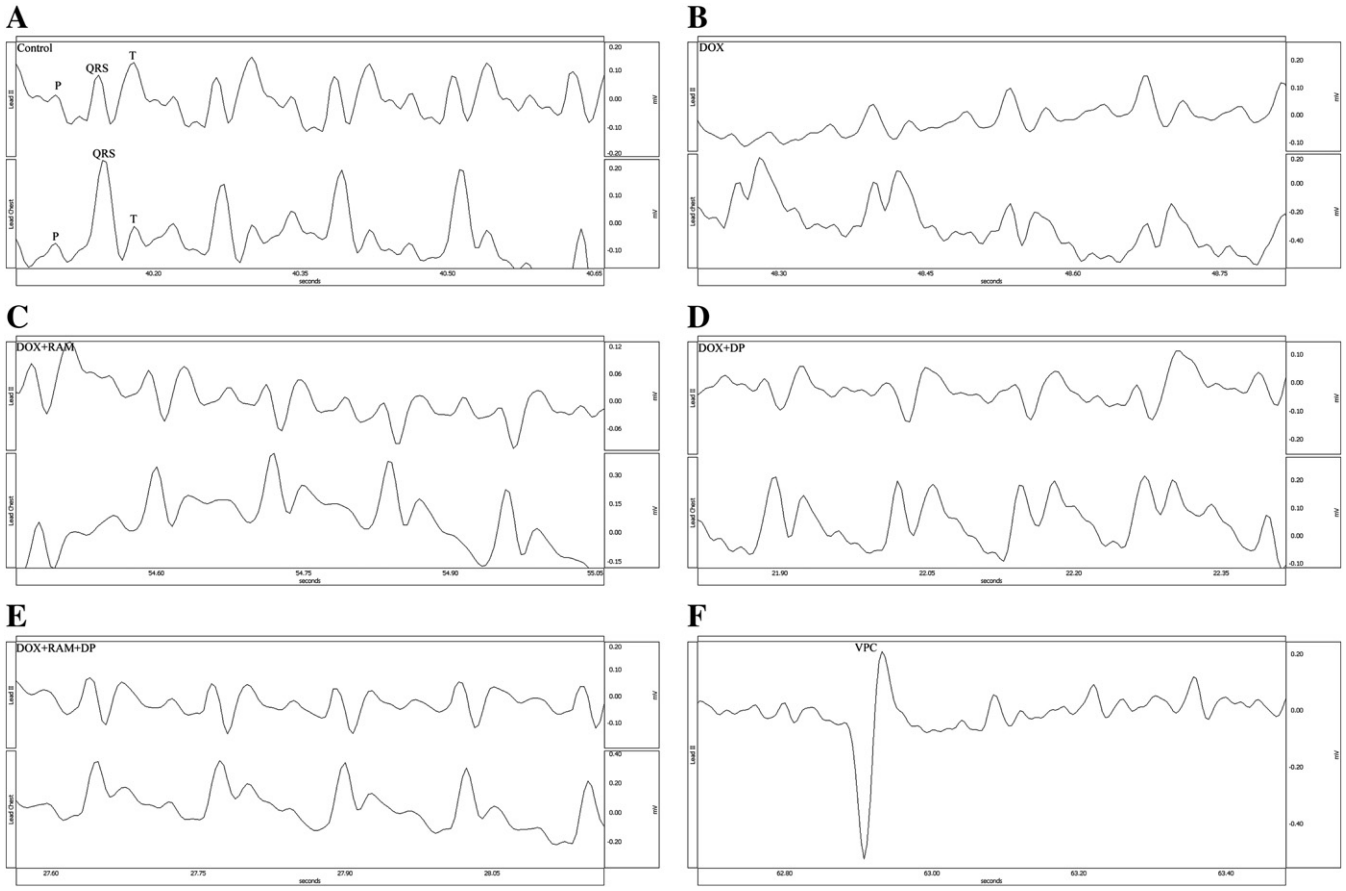

Fig. 1. The original ECG recordings using data-acquisition software in conscious rats with no anaesthetics in the groups. The ECG of each group demonstrates two leads recording for derivations in the same rat. Lead II is at the upper section and Lead Chest is at the lower section. A. Control group, B. DOX group, C. DOX + RAM group, D. DOX + DP group, E. DOX + RAM + DP group, F. The occurrence of VPC in a rat treated with DOX over Lead II.

and HTC value due to cardiotoxicity and these parameters have been ameliorated either by RAM or by DP. Levels of IL-1β and TNF-α increased after the DOX injections. RAM and DP significantly prevented the increase in IL-1β level and alleviated histopathological changes in heart tissues as compared with the DOX group. TNF-α level decreased only in RAM treatment compared to DOX group.

Darbepoetin alfa, as an erythropoiesis stimulating protein, increases tHb and erythrocyte levels. ACE inhibitors are being used in the treatment

Table 1
Parameters of ECG, Echo, haematology and cytokines in the groups (n = 8).

Parameters	Control	DOX	DOX + RAM	DOX + DP	DOX + RAM + DP
ECG					
P, ms	24.5 ± 1.4^{a}	28.8 ± 1.3^{b}	26.3 ± 0.6^{ab}	23 ± 0.9^{a}	24.3 ± 1.4^{a}
PQ, ms	39.6 ± 2.5	39.3 ± 1.3	37.3 ± 0.8	38.8 ± 0.9	40.9 ± 1.7
QRS, ms	30.6 ± 0.9^{a}	37.1 ± 1.4^{b}	29.8 ± 1^{a}	33 ± 1.3^{ab}	34.4 ± 1^{ab}
QT, ms	64.4 ± 0.7^{a}	79.9 ± 1.7^{c}	69.6 ± 1.8^{b}	72.2 ± 1.5^{b}	72.7 ± 0.8^{b}
QTc, ms	5.8 ± 0.1^{a}	7 ± 0.1^{c}	6.3 ± 0.2^{b}	6.5 ± 0.1^{b}	6.6 ± 0.1^{b}
RR, ms	123.4 ± 1.7	129.3 ± 2.5	121.9 ± 1.9	123.4 ± 2.1	122.6 ± 2
HR, bpm	487 ± 6.5	465.6 ± 8.7	493.2 ± 7.3	487.5 ± 8.7	490.6 ± 7.8
VPC	0	5	2	3	0
Echo					
LVEF, %	78.29 ± 1.19^{a}	61.29 ± 2.18^{b}	61.14 ± 1.03^{b}	63.25 ± 2.18^{b}	63.43 ± 3.66^{b}
LVFS, %	41.71 ± 1.21^{a}	28 ± 1.46^{b}	28.29 ± 0.71^{b}	30 ± 1.57^{b}	30.71 ± 2.67^{b}
Haematology					
RBC, $\times 10^{6}/\mu L$	8.6 ± 0.1^{a}	5.6 ± 0.6^{c}	7.1 ± 0.2^{b}	6.8 ± 0.3^{b}	7.3 ± 0.4^{b}
HCT, %	43.9 ± 0.6^{a}	33.1 ± 2.9^{b}	40.5 ± 1.4^{a}	38.4 ± 1.5^{a}	41.1 ± 1.3^{a}
tHb, g/L	14.7 ± 1.1^{ab}	12.2 ± 1^{a}	14.8 ± 0.5^{ab}	14.7 ± 0.7^{ab}	15.1 ± 0.6^{b}
Cytokines					
TNF-α, pg/mL	47.4 ± 4.7^{a}	134.7 ± 7.4^{c}	95.9 ± 9.2^{b}	129.6 ± 12.1^{c}	121.1 ± 9.6^{c}
IL-1β, pg/mL	33.6 ± 2.4^{a}	53.9 ± 4.1^{c}	38.9 ± 2.4^{b}	35.2 ± 3.4^{ab}	30.9 ± 2.1^{a}

Means with different superscripts are statistically different within a row (Mean ± SEM). Statistical comparison was defined by $P < 0.05$ between groups with Duncan's multiple range test. ms: millisecond; HR: heart rate; bpm: beats per minute; VPC: ventricular premature complex, number of animals seen VPC per total 8 rats LVEF: Left ventricular ejection fraction; LVFS: Left ventricular fractional shortening; RBC: red blood cells; HCT: hematocrit; tHb: total haemoglobin; TNF-α: Tumour necrosis factor alpha and IL-1β: Interleukin-1 beta.

of CHF for systolic dysfunction. The animals, induced cardiotoxicity, and have shown anaemia, systolic dysfunction and repolarization abnormalities based on the results of haematology, echocardiography and ECG, respectively. It is concluded that anaemia and systolic dysfunction are the main problems of DOX cardiotoxicity and solving these problems should be the main goals of the therapy. Therefore, administration of weekly DP injection and daily oral RAM together may have significant potentials to improve heart conduction abnormalities and anaemia but not the systolic dysfunction caused by DOX toxicity during same period of treatments.

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Management of transradial access complications in the cardiac catheterization lab

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Over the last years a dramatic transformation from the femoral to the radial access has taken place worldwide. Although the identification and management of transfemoral access complications are well studied, literature about the management of transradial access complications in the cath lab is scarce.

The hydrophilic coating rather than the sheath length seems to reduce its incidence [1]. Sheathless guide catheters, although unavailable in the US, may decrease the risk of spasm, arterial trauma or occlusion [2,3]. Inadequate sedation or vasodilatation, repeated unsuccessful access attempts, small arteries, rapid advancement and exchange of catheters and the use of multiple catheters appear to be the most common causes of spasm (Fig. 1). [4].

a) *Before sheath insertion*. Adequate systemic sedation and analgesia are necessary [5]. In the PRE-DILATE trial topical nitroglycerin with lidocaine ointment managed to increase the size of the radial artery locally [6]. Furthermore, periradial subcutaneous administration of nitroglycerine and prilocaine may improve radial pulse and access time [7]. The use of ultrasound-guided access was not superior to palpation-guided access regarding success rates or time to access [8].

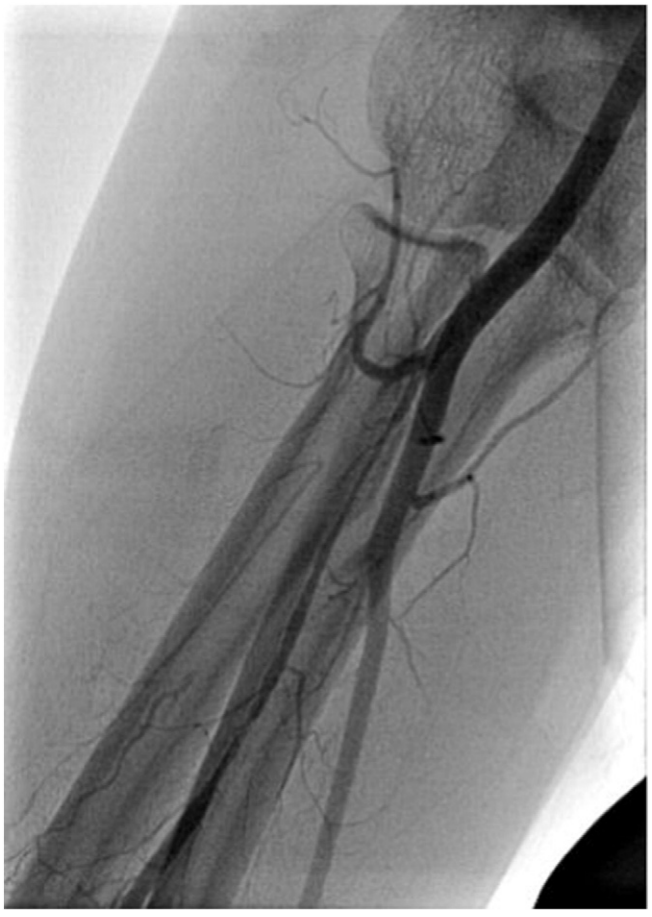

Fig. 1. Radial artery spasm.

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