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Protective effects of melatonin on doxorubicin induced cardiotoxicity in isolated rat heart

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ABSTRACT

Doxorubicin is a highly effective cancer chemotherapeutic agent and its clinical use is limited by its serious cardiotoxicity. Oxidative damage is suggested to play a major role in its cardiotoxicity. In this study we aimed to research the protective effects of melatonin-which is a free radical scavenger and antioxidant -on Doxorubicin-induced cardiotoxicity in isolated perfused rat heart. Male wistar albino rats were divided into four groups: 1.group: control (1ml (i.p) sterile saline), 2.group: Doxorubicin (Dox) (one dose, 10 mg/kg (i.p)), 3.group: Dox (one dose, 10 mg/kg (i.p)) + Melatonin (Mel) for 7 days once a day 10 mg/kg (i.p)), 4.group: Melatonin (for 7 days once a day 10 mg/kg (i.p)). After 7 days, the hearts were isolated and perfused by Langendorff system. Heart rate, coronary perfusion pressure, Left ventricular developed pressure (LVDP), LV (dP/dt)max and LV(dP/dt)min which shows max and min pressures during systole and diastole per time were recorded.

As a result; a significant increase in coronary perfusion pressure and LV(dP/dt)min and also a significant decrease in LVDP, LV(dP/dt)max and heart rates in the Dox group according to control group showed altered cardiac functions induced by Dox. versus the Dox group, in the Dox+Mel group the coronary perfusion pressure and LV(dP/dt)min significantly decreased and LVDP and LV(dP/dt)max significantly increased. So it is concluded that melatonin has protective effects on doxorubicin induced cardiotoxicity characterized with altered heart contractility and hemodynamics.

Key Words: Isolated heart, Langendorff, doxorubicin, cardiotoxicity, melatonin

Introduction

Doxorubicin is one of the effective antineoplastic drugs, is commonly used against breast, ovarian, testicular, thyroid, lung cancers and hematological cancers including Hodgkin Lymphoma and prevalent non-Hodgkin lymphomas (1). However, it has serious cardiotoxic side effects which is limiting its clinical use (2). The main important problem is this toxic side, reducing quality of life and sometimes causing fatalities. For this reason, different methods are currently being tried to reduce cardiotoxic effects in antracycline treatment. Since an approach that involves reducing the maximum cumulative dose of the antracyclines would also reduce theraupetic effects, different methods like the synthesis of low cardiotoxic antracycline analogues, using different administration methods, encapsulating these drugs in different formulations like liposomes were tried for this purpose. The mechanism of this cardiotoxicity was researched by many studies and today it is thought that the major cause of this effect is the tissue damage induced by free oxygen radicals (2). During the doxorubicin biotransformation, reduction of the kinone group by cytochrome P-450 reductase and xanthine oxidase into the semikinone radical (3) and the capture of the electrons released during this process by oxidative agents like oxygen initiates a reaction chain that forms free oxygen radicals and causes cardiomyocytes cell death (4,5). The hydrojen peroxide and superoxide radical reduce the levels of the enzyme endogen glutation peroxidase that is responsible for scavenging free radicals, increase oxidative stress and it is resulted with cardiomyopathy (6,7).

When it is discovered that the formation of free oxygen radicals have a significant effect in the cardiotoxical mechanism of doxorubicin, due to the decrease of indigenous antioxidant systems in the body, exogenous antioxidants were used as well (5,8). Many drugs, especially those with free radical scavengers and antioxidant effects, were used within the combinations (9-11). Melatonin, that is physiologically present in the body and secreted by the pineal gland, is among the antioxidant agents that were used to protect against the oxidative damage. Both in vitro and in vivo studies show that melatonin and its

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metabolites are strong free oxygen radicals scavengers (12,13). It can protect macromolecules, especially DNA, against the oxidative damage caused by free oxygen radicals, especially the highly toxic hydroxyl radicals (14). Radicals that damage the DNA, activate poly-ADP-ribose synthase (PARS), which is a nuclear enzyme in the cell. This enzyme is activated by the breaking of a single DNA chain and initiates necrotic cell death by triggering very high energy consumption in the cells. It was reported that melatonin can inhibit PARS activity and prevent organs damage in shock, inflammation and ischemia/reperfusion (I/R) (15).

In our study, isolated perfused heart model was used, which allows the observation of the acute symptoms of cardiomyopathy induced by doxorubicin and the changes in heart functions. It is aimed to investigate protective effects of melatonin by examining the changes in contractility and hemodynamics of the heart that was perfused by using the Langendorff system (16).

Materials and methods

Experimental animals used: 40 adult wistar albino rats weighing 250-300 grams were used that were procured from Dicle University Health Sciences Applications and Research Center (DUSAM) with the approval of the ethics board. During the study, the rules for Protection of Animal Rights were carefully followed.

Pharmacological analysis: The study was performed with 4 groups of 10 animals each.

1. Control Group; received the injection of 1ml (i.p) sterile saline.
2. Doxorubicine (Dox) Group; single dose of 10mg/kg (i.p) Dox. injection. Hearts were isolated at the end of day 7.
3. Dox + Melatonin (Dox+Mel) Group; single dose of 10mg/kg (i.p) Dox. injection. Also received melatonin injections of 10mg/kg (i.p) once a day at the same time for 7 days.
4. Melatonin Group (Mel); melatonin injections of 10mg/kg (i.p) once a day at the same time for 7 days.

In all groups rats received 100mg/kg Ketamine+15mg/kg Xylasine intramuscularly (i.m) as anesthetic. The rats were administered Heparin (500IU/kg) from the femoral vein to prevent any coagulation during surgical operation; the thorax was opened via right side sternotomy, and the hearts were isolated by ascendant aorta. The hearts were left in iced Krebs solution and cannulated from the aorta using a short cannula and attached to the Langendorff system. Coronary perfusion was achieved by using the Krebs solution (aspirated with 5% CO_2 and 95% O_2 mix) with constant flow. Perfusion was performed using a peristaltic pump. The coronary perfusion pressure (PP) was measured with a pressure transducer connected to the aortal infusion cannula. Latex balloon connected to a polyethylene catheter was placed in the left ventricular through the mitral valve in the left atrium. Catheter with a second pressure transducer was filled with distilled water and the latex balloon at the tip of the catheter was inflated using the distilled water, applying a pressure of 5-6 mmHg. After the 30-45 minute stabilization period that is required to reach maximum cardiac function values, the Left Ventricular Developed Pressure (LVDP) was measured in the left ventricular with the latex balloon. Also the LV(dP/dt)max and LV(dP/dt)min values, that indicate the maximum systolic and minimum diastolic pressure of the left ventricular were recorded as an indication of the contraction power of the heart. In addition, the heart rate (HR) which indicates the number of heart rates per minute was recorded using electrodes attached to the hearts. In all groups, recordings taken by Biopac MP 30 amplifier were analyzed on the computer.

Statistical analysis: Statistical Package for the Social Sciences (SPSS) software was used to compute statistical data. All results were expressed as means ± standard deviation and Kruskal-Wallis test was used as the variance analysis to determine the differences between groups. Mann-Whitney U-test was used for comparisons of differences between two independent groups. A p value < 0.05 was considered statistically significant.

Results

When Mann-Whitney U-test was used for comparisons of differences between two independent groups (Table 1); the coronary perfusion pressures significantly increased in the Dox group according to control group ($p<0.001$). It was determined that the increased perfusion pressure was significantly decreased in the Dox+Mel group according to the Dox group ($p=0.001$). While no significant difference in coronary perfusion pressure was observed in the Mel group, according to the control group

Table 1. Data collected from the groups (the results are shown as Arithmetic mean (X) ± standard deviation (SD)

	Control (a)	Dox (b)	Dox-Mel (c)	Mel (d)	p
PP (mmHg)	71.34 ± 14.07	126.02 ± 16.67	77.37 ± 27.47	78.40 ± 12.30	(a-b) (b-c) p<0.001, (b-d) p<0.05
HR (beat per min)	280.90 ± 16.71	213.00 ± 27.44	225.90±40.61	279.30±16.15	(a-b) (b-d) p<0.001, (a-c) (c-d) p<0.05
LVDP (mmHg)	91.66 ± 6.83	53.96 ± 11.02	70.09 ± 7.55	90.92 ± 6.80	(a-b) (a-c) (b-d) (c-d) p<0.001, (b-c) p<0.05
LV(dP/dt)max	1351.80 ± 72.98	914.90 ± 77.9	1023.90±54.3	1299.50±73.7	(a-b) (a-c) (b-d) (c-d) p<0.001, (b-c) p<0.05
LV(dP/dt)min	-1142.00 ± 58.04	-764.60 ± 65.79	-1037.00±62.3	-1122.30±74.9	(a-b) (a-c) (b-c) (b-d) p<0.001, (c-d) p<0.05

(p>0.05), a significant difference similar to the control group was observed according to the Dox group (p<0.001) (Figure 1).

Heart rates were significantly decreased in the Dox group according to the control group (p<0.001), and there was no significant difference with the Dox+ Mel group (p>0.05). While no difference was observed in the Mel group according to the control group (p>0.05), a significant difference was observed according to Dox and Dox+Mel groups (p<0.001, p<0.05) (Figure 2).

When LVDP responses are checked, it was determined that there's a significantly decrease in the Dox group according to the control group (p<0.001). This decrease in the Dox group was observed significantly increase in the Dox+Mel group (p<0.05). While no significant difference was observed in the Mel group, according to the control group (p>0.05), a significant difference was observed according to Dox and Dox+Mel groups (p<0.001) (Figure 3).

LV(dP/dt) min significantly increase in the Dox group according to the control group (p<0.001). This increase significantly decreased in the Dox+Mel group (p<0.001) and in the Mel group, while no significant difference was observed according to the control group (p>0.05), there's a significant decrease according to Dox (p<0.001) and Dox+Mel groups (p<0.05) (Figure 4).

LV(dP/dt) max significantly decrease in the Dox group according to the control group (p<0.001). This decrease in the Dox group whereas significantly increase in the Dox+Mel group (p<0.05). Similarly to the LVDP responses, while no significant difference was observed in the Mel group according to the control group (p>0.05), a significant difference was observed according to Dox and Dox+Mel groups (p<0.001) (Figure 5).

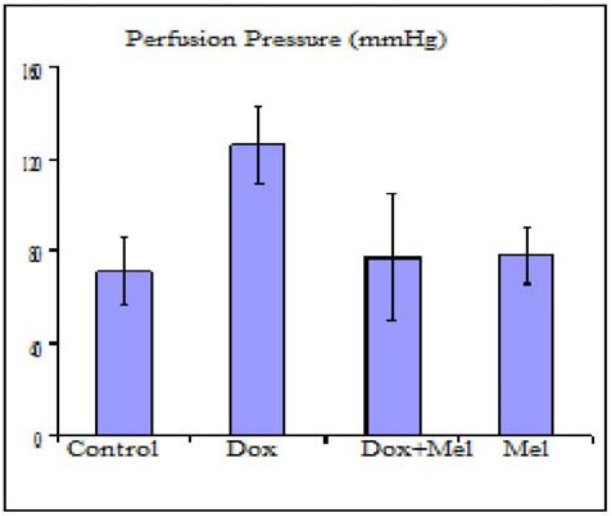

Fig. 1. Perfusion pressure in all groups (mmHg).

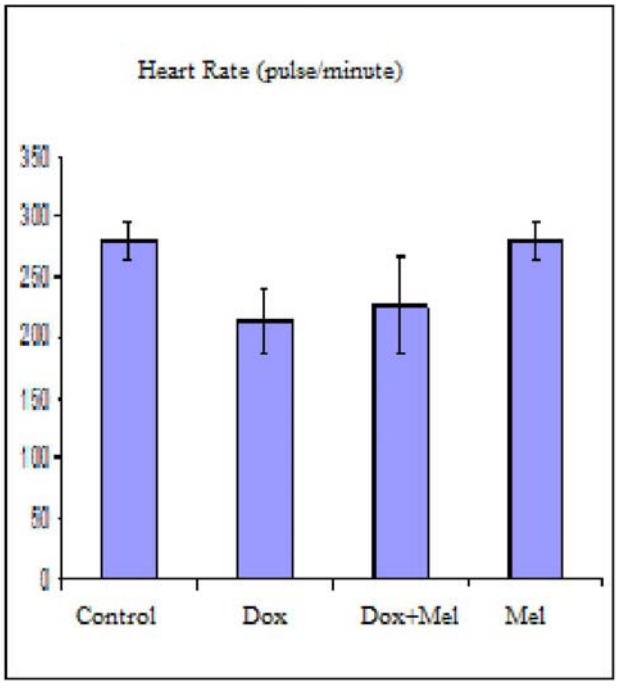

Fig. 2. Heart Rate (beat per min).

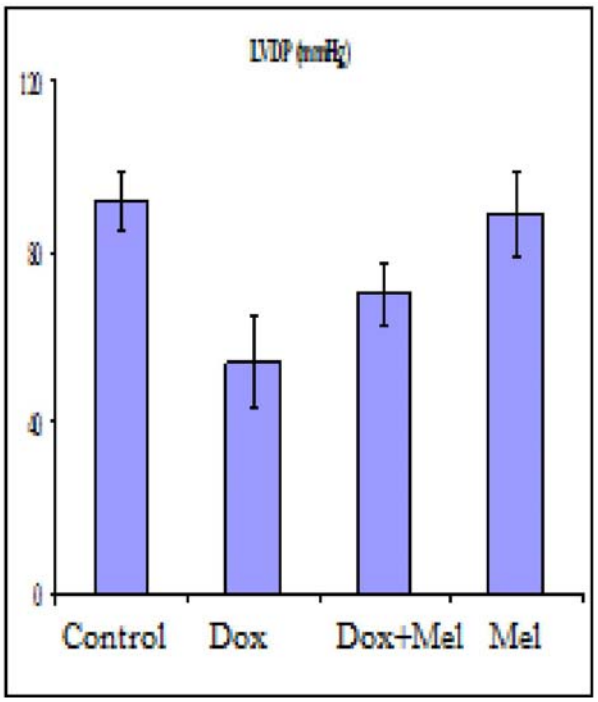

Fig. 3. Left Ventricular Developing Pressure (LVDP) (mmHg).

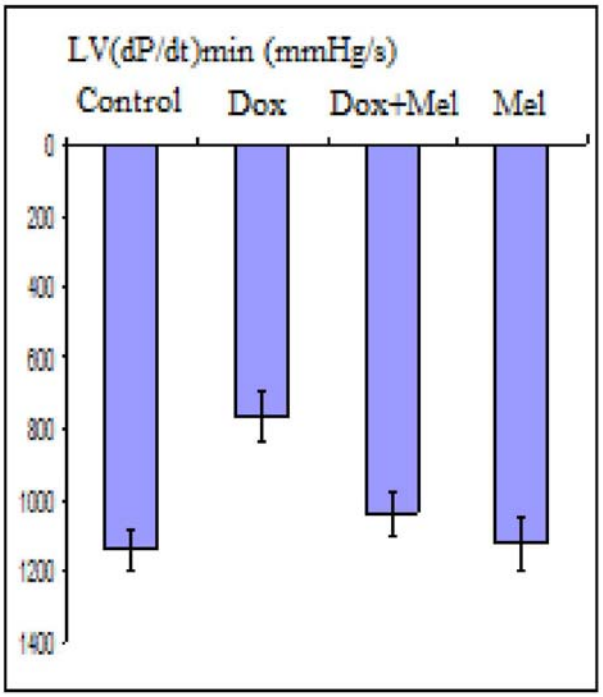

Fig. 4. Left ventricular minimum diastolic LV(dP/dt) min (mmHg/s).

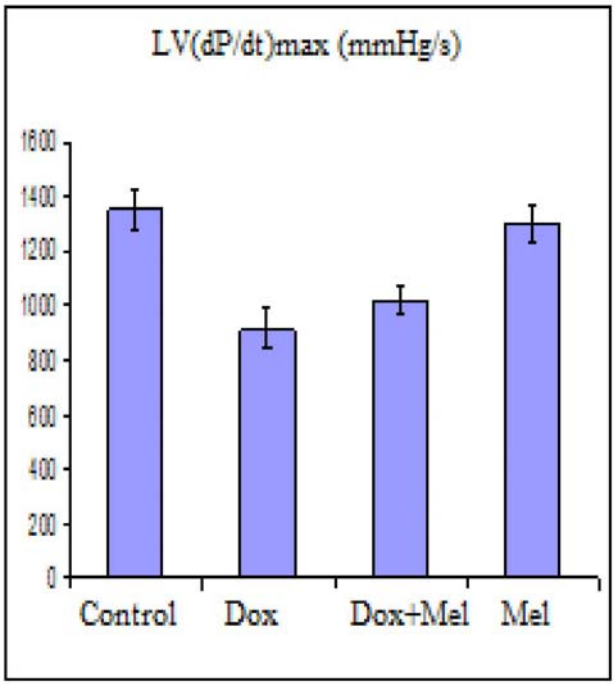

Fig. 5. Left ventricular maximum systolic pressure LV(dP/dt)max (mmHg/s).

Discussion

Melatonin, that we used to demonstrate its protective effects against the cardiac dysfunction induced by Doxorubicin, has strong antioxidant and direct free radical scavening effects, as shown by several studies in recent years (17). Our results have shown that in Dox+Mel group there was a significant increase in the contractile functions and there was a significant decrease in coronary perfusion pressure, according to the Dox group. These findings demonstrate that the

cardiomyopathy symptoms induced by doxorubicin is prevented by melatonin. It was also observed that in Dox+Mel group Left ventricular pressure (LVDP) and maximum systolic pressure per second ($LV(dP/dt)_{max}$) were significantly increased according to the Dox group, while there was a significant decrease in the minimum diastolic pressure ($LV(dP/dt)_{min}$). This means left ventricular failure and heart hemodynamics disruption that develop in Dox group are prevented by melatonin.

Cardiotoxic model induced with doxorubicin injection with a dose of 10 mg/kg, which we performed in our study was reported to induce cardiotoxicity in rats (18). It was observed that there was a significant decrease in contractile functions of the hearts in the doxorubicin-injected group (18). Due to the increased adrenergical activity in the vasculature and mainly due to free oxygen radicals, a significant increase in perfusion pressure was observed (19). These findings match with the symptoms of cardiomyopathy reported to be induced by doxorubicin (3). It was also observed that when the left ventricular pressure (LVDP) and maximum systolic pressure per second ($LV(dP/dt)_{max}$) of the rats in doxorubicin groups were measured, these values show a significant decrease according to the control group, while there is a significant increase in the minimum diastolic pressure ($LV(dP/dt)_{min}$). These findings demonstrate that left ventricular failure and heart hemodynamics disruption which develop in doxorubicin treated rats, consistent with the findings of the study performed by De Nigris et al. (20) with beta blocker effective-nebivolol.

Histopathological and biochemical studies also report that the cardiac damage induced by doxorubicin can be prevented with low pharmacological doses of melatonin and melatonin can regulate arterial tonus in the cardiovascular system (21). Another study reports that melatonin reduces the cardiac toxicity of doxorubicin, and it provides this effect by inhibiting lipid peroxidation and increasing the antioxidant enzyme activity (22). Biochemical studies show that melatonin scavenges radicals like $O_2^{\cdot-}$, $OH^{\cdot}$, peroxynitrites (ONOO-) and H_2O_2 and increases the expression of antioxidant enzymes like SOD, CAT, GSH-Px and glutation reductase and inhibits the inducable nitric oxide synthase (iNOS) enzyme that can cause the increase of peroxynitrides (23,24). It was reported that due to these antioxidant speciality, melatonin has protective effects by reducing MDA levels that increase during I/R damage in various tissue and organs and nephrotoxicity induced by many antineoplastic drugs and by increasing the levels of antioxidant enzymes (25).

As a result, in our study, hemodynamical and functional changes were observed in isolated perfused hearts of doxorubicin pretreated rats and it was shown that the symptoms characterized with the left ventricular failure are prevented by melatonin and it is also concluded that measuring the heart functions by Langendorff will be complementary to histopathological and biochemical studies.

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