Protective effect of paracetamol in doxorubicin-induced cardiotoxicity in ischemia/reperfused isolated rat heart

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Abstract

Objective: Doxorubicin (DOX) induces cardiac dysfunction. Paracetamol (APAP) has also been established as an effective cardioprotective agent during ischemia/reperfusion. Therefore, this study aims to evaluate the effect of APAP on DOX-induced cardiotoxicity in ischemia/reperfused isolated rat heart.

Methods: A total of 36 rats were equally divided into four groups: control, DOX (30 min, 20 μM DOX perfusion), APAP (15 min before and after ischemia, 0.35 mM APAP perfusion), and DOX+APAP (perfused with the same protocol in DOX and APAP groups). The isolated hearts were perfused according to the Langendorff method. Cardiac parameters, including left ventricular developed pressure (LVDP), heart rate (HR), coronary flow (CF), and rate pressure product (RPP; LVDP×HR) were measured. Lactate dehydrogenase (LDH) concentration was also assessed.

Results: At the end of the baseline period, the RPP, HR, and CF values were lower in the DOX group than in the control group ($p<0.01$). Meanwhile, there were no significant differences between the values of cardiac function parameters in the DOX+APAP and control groups. In the reperfusion period, the RPP and CF values were significantly increased in the DOX+APAP group compared with the DOX group ($p<0.05$). Furthermore, the LDH concentration was decreased in the DOX+APAP group compared with the DOX group.

Conclusion: APAP perfusion protected the hearts against DOX-induced cardiotoxicity in the baseline and ischemia/reperfusion conditions. These findings can be explained by the effect of APAP on antioxidant capacity and mitochondrial permeability transition pores.

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Keywords: paracetamol, doxorubicin, cardiotoxicity, isolated heart, ischemia/reperfusion

Introduction

Doxorubicin (DOX), an anthracycline antibiotic sold under the trade names Adriamycin among others, has been widely used in the treatment of cancer. The antineoplastic application of DOX is limited by its common side effects, including cardiac dysfunction and fibrosis (1, 2). DOX may contribute to cardiotoxicity in many ways, including enhancement of mitochondrial calcium and reactive oxygen species (ROS), cell necrosis, and induction of proapoptotic signaling pathways (3, 4). The best described major mechanism through which doxorubicin injures the myocardium is the induction of free radical production (5-7). Mitochondrial dysfunction plays a role in the development of DOX-induced cardiotoxicity (8).

Paracetamol (acetaminophen, APAP, N-acetyl-p-amino-phenol), is a popular nonsteroidal anti-inflammatory drug, which has historically been used as an analgesic-antipyretic agent (9). In recent investigations, APAP has been established as an effective cardioprotective agent during ischemia/reperfusion (I/R), hypoxia/reoxygenation, experimentally induced myocardial infarction (10-16), and oxidative challenge (12, 14, 17, 18). Acute acetaminophen treatment also protects from myocardial infarction in a canine model (11). Acetaminophen provides significant functional and structural protection to an ischemic-reperfused myocardium; the mechanism of cardio protection seems to involve production attenuation of both hydroxyl radicals and peroxynitrite (17). It is assumed that the antioxidant properties of paracetamol conveys through its phenolic structure are part its mechanism of action (19).

Many preventive and therapeutic strategies have been explored to counteract DOX-induced toxicity and heart dysfunction (20, 21). The application of drugs with free radical scavenging properties can provide solution for doxorubicin-induced cardiotoxicity. Considering the prevalence of cancer and cardiotoxicity caused by doxorubicin, paracetamol as an over-the-counter drug with antioxidant properties can be used to prevent

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cardiotoxic effect of doxorubicin. Therefore, the current study aims to investigate the effect of paracetamol on cardiac function following doxorubicin perfusion in the I/R condition.

Methods

This experimental animal study was designed to evaluate the effect of paracetamol on DOX-induced cardiotoxicity in ischemia/reperfused rat heart.

Drugs and chemicals

Pentobarbital sodium was obtained from Sigma-Aldrich (Munich, Steinheim, Germany). Lactate dehydrogenase (LDH) was assessed using a cytotoxicity detection kit (Roche, Mannheim, Germany). Doxorubicin hydrochloride was purchased from Pharmacia (Milan, Italy), paracetamol from Cobel Darou (Tehran, Iran), and all other chemicals from Merck (Darmstadt, Germany).

Animals

The experimental protocol was reviewed and approved by the Ethics Committee of the Kermanshah University of Medical Sciences, and all animals used in the study received humane care in compliance with institutional animal care guidelines. Male Wistar rats weighing 250–300 g were housed in groups of three per cage, under controlled conditions of light (12-h light/dark cycles), temperature (22°C±3°C), and relative humidity (24%±6%), with free access to food and water.

Experimental protocols and animal grouping

The animals were anesthetized with pentobarbital sodium (60 mg/kg intraperitoneally). Their hearts were excised and immediately arrested in ice-cold Krebs solution and rapidly cannulated and retrogradely perfused through the aorta in a noncirculating Langendorff apparatus. The hearts were perfused with Krebs–Henseleit buffer (KHB), which contained sodium chloride (118 mmol/L), sodium bicarbonate (25 mmol/L), potassium chloride (4.8 mmol/L), potassium dihydrogen phosphate (1.2 mmol/L), magnesium sulfate (1.2 mmol/L), glucose (11 mmol/L), and calcium chloride (1.2 mmol/L) at 7.4 pH (22, 23). The buffer was bubbled with 95% O_2 and 5% CO_2 at 37°C. Perfusion was performed under a constant hydrostatic pressure of 60 mm Hg. Following the removal of the left atrial appendage, a deflated water-filled latex balloon was inserted through the mitral valve into the left ventricle. This balloon was connected to a pressure transducer (MLT 844; AD Instruments, New South Wales, Australia), which was connected to a computer by a power lab (model ML825; AD Instruments, New South Wales, Australia) for cardiac performance monitoring. At the beginning of the experiment, the balloon volume was adjusted to achieve a stable end-diastolic pressure of 5–10 mm Hg (22, 23). Different hemodynamic parameters were assessed, including heart rate (HR; beats/min) and left ventricular developed pressure (LVDP; mm Hg), which

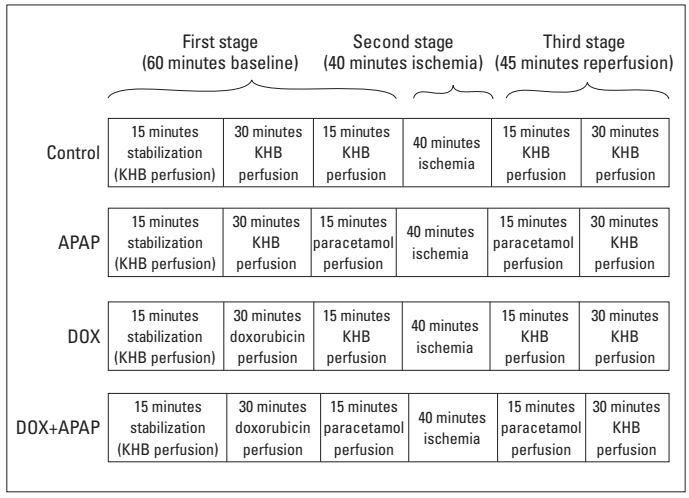

Figure 1. Schematic protocol. Based on the experimental group, isolated hearts were perfused with APAP: (0.35 mM paracetamol, 15 min before and 15 min after ischemia), and/or DOX: (20 µM doxorubicin, 30 min at baseline)

was defined as the difference between peak systolic and end-diastolic pressures. The rate pressure product (RPP) was calculated as LVDP×HR. Coronary flow (CF; mL) was also measured by volumetric collection of coronary effluent per minute. Thirty-six rats were randomly assigned to four groups of nine rats each. As shown in Figure 1, all the hearts were subjected to 60-min baseline then 40-min global normothermic ischemia followed by 45-min reperfusion period. In the controls, the hearts were perfused with KHB. In the APAP group, the hearts received KHB-contaning APAP (0.35 mM) (13, 14) in the last 15-min of the baseline and the first 15-min of the reperfusion periods. In the DOX group, after stabilization, the hearts received KHB-contaning DOX (20 µM) (24) for 30 min in the baseline period. In the DOX+APAP group, the hearts received 20 µM DOX in the first 30-min of the baseline period and APAP (0.35 mM) in the last 15-min of the baseline and the first 15-min of the reperfusion periods. Global normothermic ischemia was induced by clamping the aortic cannula and immersing the hearts in KHB at 37°C. The I/R injury level was determined based on the time until the onset of ischemic contracture (time to contracture start), maximum contracture during ischemia, cardiac function recovery, and the release of lactate dehydrogenase (LDH) into the coronary effluent at the onset of reperfusion. According to the literature, time to contracture start is the moment at which the end-diastolic pressure starts to increase during ischemia (in minutes). Maximum contracture was also considered as the maximum rise in diastolic tension after the onset of ischemia.

LDH assessment

The LDH content of CF reperesents the extent of I/R injury. Therefore, CF was collected at the first minute of reperfusion. To measure LDH, a cytotoxicity detection kit and known quantities of LDH (Sigma-Aldrich Chemie GmbH, Munich, Steinheim, Germany) as the standards were used.

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Table 1. The effect of paracetamol and doxorubicin on myocardial function of isolated rat hearts before and after exposure to 40-min global normothermic ischemia

Parameters and periods	Control (n=9)	APAP (n=9)	DOX (n=9)	DOX+APAP (n=9)
Baseline values (15th min)				
LVDP	83±3	82±3	93±3	85±3
HR	289±14	288±13	268±6	304±15
CF	13.1±0.24	12.6±0.11	12.4±0.2	13±0.16
RPP	23976±930	23050±115	24841±816	25685±805
Baseline values (45th min)				
LVDP	89±4	83±3	70±1**	73±2**
HR	275±12	277±11	220±9**	239±6
CF	12.2±0.24	12.3±0.15	9.5±0.39***	9.6±0.3***
RPP	23947±114	23062±132	15536±530***	17530±232***††††
Baseline values (60th min)				
LVDP	91±4	86±3	82±1	82±3
HR	267±10	260±9	222±9*	257±7
CF	12.5±0.07	12.5±0.21	10.7±0.21***	11.35±0.25
RPP	23613±968	22359±100	18326±878**	21136±538
Reperfusion (45th min)				
LVDP	37±5	44±5	24±3	38±4
HR	235±14	226±14	185±10*	215±11
CF	6.4±0.26	6.9±0.24	4.5±0.3***	5.9±0.15##†
RPP	8870±331	9966±926	4573±465**	8173±133#

CF- coronary flow, mL/min; HR- heart rate, beats/min; LVDP- left ventricular developed pressure, mm Hg; LVDP×HR, RPP- rate pressure product. Data sets were analyzed by analysis of variance (ANOVA) followed by Tukey's post hoc test and expressed as mean±SEM. # or * or † $P<0.05$, ## or ** or †† $P<0.01$, ### or *** or ††† $P<0.001$. Significant differences versus the control, DOX, and APAP groups are shown by symbols *, #, and †, respectively.

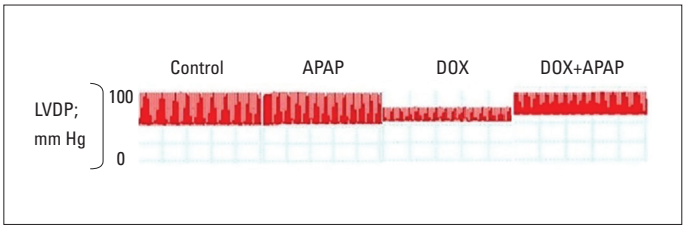

Figure 2. Power lab recorded graphs demonstrates traces of left ventricular develop pressure (LVDP) in four groups at 45th min of reperfusion

Statistical analysis

All data are expressed as mean±standard error of mean (SEM); the Kolmogorov–Smirnov test was applied to test normality of distributions. One-way ANOVA was applied and Tukey post hoc test was used for multiple comparisons as offered by SPSS version 20. P<0.05 was considered statistically significant.

Results

Hemodynamic function

Hemodynamic data were recorded throughout the 60-min baseline, 40-min ischemia, and 45-min reperfusion periods. Table 1 summarizes the hemodynamic parameters of isolated rat hearts in each group in the baseline and reperfusion periods.

There were no significant differences between the hemodynamic parameter values in the four groups in the first 15-min of the baseline period (stabilization). The hemodynamic parameters, including RPP, LVDP, CF, and HR were constant throughout the baseline period in the control group. At the 45th min of the baseline (after receiving DOX), cardiac parameters including LVDP, RPP, and CF were significantly reduced in the DOX and DOX+APAP groups compared with the control group (p<0.01) (Table 1). In addition to the mentioned parameters, the HR value was significantly reduced in the DOX group compared with the control group (p=0.003). At the end of the baseline period (60th min), the values of RPP, HR, and CF in the DOX group were still lower than those in the control group (p<0.01). Meanwhile, there were no significant differences between the values of cardiac function parameters in the DOX+APAP and control groups.

At the end of the reperfusion period, the values of RPP, HR, and CF in the DOX group were significantly lower than those in the control group (p<0.05). In the DOX+APAP group, the values of RPP and CF significantly increased compared with those in the DOX group (p<0.05 and p<0.001, respectively); however, the value of CF was still lower than that in the control and APAP groups (p<0.05).

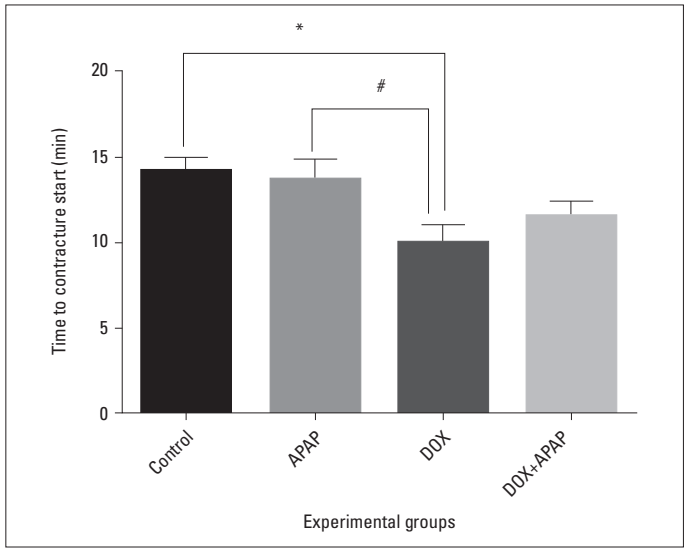

Figure 3. Time to contracture start (minute) in experimental groups during ischemia. Data are expressed as Mean±SEM. *P=0.018, and #P=0.042

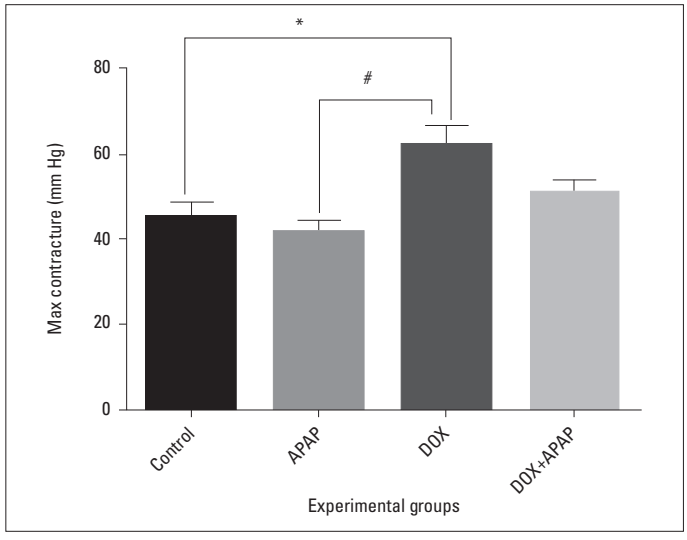

Figure 4. The maximum contracture (mm Hg) in experimental groups during ischemia. Data are expressed as Mean±SEM. *P=0.005 and #P=0.001

Figure 2 shows a typical graph of LVDP recorded by power lab. It illustrates the differences between the experimental groups, which was described in Table 1. As represented in Figure 3, the time to contracture start in the DOX group (9.91±1.02 min) was significantly lower than that in the control and APAP groups (14.12±0.84 and 13.64±1.12 min, respectively) during ischemia. Furthermore, the maximum contracture of the hearts in the DOX group (62.06±4.37 mm Hg) was significantly higher than the values of the corresponding parameter in the control and APAP groups (45.43±3.15 and 41.70±2.23 mm Hg, respectively) (Fig. 4).

Lactate Dehydrogenase assessment

The extent of reperfusion injury in the experimental groups was determined based on the release of an intracellular enzyme into the coronary effluent. The concentration of released LDH during the first minute of reperfusion is shown in Figure 5. As represented, the amount of LDH released from the hearts of the DOX group (18.6±0.72 mU/mL) was significantly higher than that of the control (12.7±40 mU/mL), APAP (14.05±0.62 mU/mL), and DOX+APAP (12.6±0.51 mU/mL) groups ($p<0.05$).

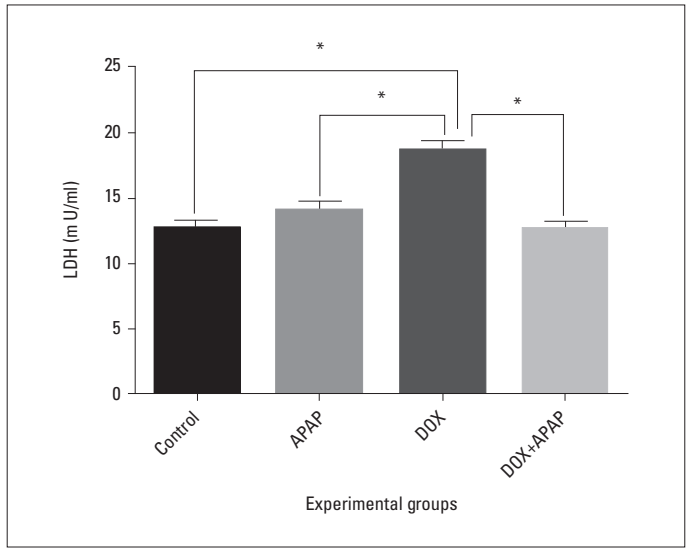

Figure 5. The concentration of released LDH during the first minute of reperfusion in the experimental groups. Data sets are analyzed by analysis of variance (ANOVA) and expressed as Mean±SEM. *P<0.001

Discussion

In the current study, 0.35 mM paracetamol was perfused to isolated hearts, 15 min before and after ischemia to investigate its probable protective effects against DOX-induced cardiotoxicity. The results showed that APAP perfusion attenuates DOX-induced cardiotoxicity and increases cardiac function parameters deteriorated by this drug at the baseline and after I/R. Furthermore, these results indicate the cardioprotective effect of APAP against adverse effects of DOX perfusion in isolated heart model.

According to our findings, 30-min DOX (20 μM) perfusion at baseline caused cardiac damage and reduced cardiac function parameters including RPP, LVDP, HR, and CF compared with control. Furthermore, reduction of time to contracture start and enhancement of maximum contracture showed exacerbated cardiac ischemic injury due to the adverse effects of DOX in the ischemic period. These results are consistent with other studies reporting a decline in cardiac function parameters after DOX administration (24, 25).

According to the results obtained by APAP perfusion, there were no statistically significant differences between the cardiac function parameters of control and APAP-perfused hearts at baseline (Table 1), which is consistent with the Merrill report in 2002 (12). However, 15-min APAP perfusion following 30-min DOX perfusion improved DOX-induced cardiac dysfunction at the 60th min baseline in the DOX+APAP group. Thus, unlike

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the DOX group, there was no significant difference between the DOX+APAP and control groups. In comparison to the value of RPP at the 15th min baseline, the value of RPP at the 60th min baseline declined by 27% in the DOX group and by 18% in the DOX+APAP group. This improvement reveals the protective effects of APAP perfusion in the DOX+APAP group in the baseline period. Furthermore, prolongation of time to contracture start and reduction of maximum contracture in the DOX+APAP group in ischemic condition represent the protective effects of 15-min APAP perfusion before ischemia.

Similar to the baseline period, the results of postischemic period showed that DOX perfusion reduced cardiac function parameters including RPP, HR, and CF compared with control. These results showed an exacerbation of cardiac I/R injury following DOX perfusion. This was consistent with previous reports indicating DOX-induced cardiotoxicity and exacerbated cardiac I/R injury (24, 25). Based on previous reports, several mechanism are responsible for cardiotoxicity of DOX, including increased reactive oxygen species (ROS), inhibition of oxidative phosphorylation (26-29), mitochondrial dysfunction, and induction of mitochondrial permeability transition pore (MPTP) opening (8, 25, 29, 30).

The perfusion of paracetamol before and after the ischemic period improved cardiac function parameters in the DOX+APAP group at the end of reperfusion. According to the results, the values of RPP and CF in the DOX+APAP group not only reached the control state but were also significantly higher than those in the DOX group. Improvement in these parameters shows the preventive role of APAP against DOX-induced cardiotoxicity. As represented in Figure 5, the LDH concentration in DOX group was significantly higher than that in other groups, thus revealing an exacerbation of cardiac ischemic injury due to DOX perfusion. On the other hand, the LDH concentration reduced in the DOX+APAP group and was even significantly lower than that in the DOX group. These results represent the cardioprotective effect of APAP against the adverse effects of DOX in ischemia reperfusion condition. Previous studies have also reported the positive effects of APAP in cardiac function in other models (11, 12, 14). For example, the cardioprotective effect of both chronic and acute acetaminophen treatment (0.35 mM) following I/R in an isolated perfused guinea pig myocardium have been previously reported (11, 12). Another study has demonstrated that APAP perfusion can protect the heart against I/R injury by antioxidant properties (14). It has also been reported that acetaminophen mediates the attenuation of both hydroxyl radicals and peroxynitrite production following I/R (13, 14), inhibits MPTP opening and mitochondrial swelling following myocardial I/R (19), and inhibits lipid peroxidation (31, 32). The overall protective effects of APAP against DOX-induced cardiotoxicity are probably due to its effects on antioxidant capacity.

Schunke et al. (33) have revealed that APAP attenuates DOX-induced cardiac fibrosis via negative regulation of collagen synthesis and extracellular matrix deposition. In the current study, APAP was used in an isolated heart model to investigate its effects against DOX perfusion, and the findings showed that APAP perfusion has protective effects against DOX-induced cardiotoxicity at both baseline and reperfusion periods. This is the first time that we described the ability of paracetamol as a common and inexpensive drug to protect the heart against DOX-induced cardiotoxicity and preserve cardiac function before and after ischemia in an isolated rat heart. The protective role of paracetamol in the current study might be explained by different mechanisms, including APAP antioxidant potency and mitochondrial preservation.

Study limitations

The limitation of our study was that we did not perform histopathological examinations and mitochondrial function and oxidative stress assessment in cardiomyocytes. Further studies are required to elucidate their precise roles.

Conclusion

In the current study, paracetamol perfusion improved the baseline and postischemic cardiac function parameters in DOX-perfused hearts. Therefore, paracetamol as a common analgesic-antipyretic agent could be used to reduce the cardiotoxic effects of doxorubicin. It needs to be further investigated in future studies.

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