Decreased Myocardial Tl-201 Uptake in Rats: Early Sign of Doxorubicin Induced Myocardial Damage and the Relation to Inflammation

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ABSTRACT

***Aim**: In the present study, we demonstrated that total cardiac 201Tl uptake changes associated with histological findings in DOX-induced early myocardial injury.*

***Method**: Early DOX cardiotoxicity was induced in normal rats by giving 15 mg/kg DOX intraperitoneally. Cardiac uptake studies and the blood sampling for creatine kinase (CK) and lactate dehydrogenase (LDH) assay has been performed on the 3rd (acute phase) and 16th days (subacute phase) after the treatment, respectively. Rats were killed by heart puncture and the hearts removed by dissection at 60 min after the injection of 7.4 MBq 201Tl. The ratio of total cardiac uptake to the injected dose (%ID/g x BW, where ID is injected dose and BW is body weight) was calculated.*

***Result**: DOX led to a significant decrease in myocardial uptake of 201Tl in both treatment groups ($p<0.05$). There was no significant difference in the %ID/g x BW between acute and subacute phases ($p>0.05$). DOX induced a significant increase in the levels of CK and LDH in serum, indicating its early cardiotoxicity ($p=0.01$). DOX treatment produced disorganization of myocardial fibers, vacuolation of the cardiac myocytes and myocardial necrosis ($p=0.01$). These cardiomyocyte injuries were accompanied by increased numbers of mononuclear cells ($p<0.05$). LDH, CK, cardiyomyopathy and mononuclear cell infiltration scores were not found significantly different between acute and subacute phases ($p>0.05$).*

***Conclusion**: The DOX-induced cardiac injury at early stage can be evaluated by 201Tl and the findings may be associated with the myocardial inflammation. Due to the complicated mechanism of DOX injury, we believe that the development process of cardiac injury and the pathological findings should be taken into consideration in interpreting the radiopharmasotic studies to be conducted for the evaluation of the early and late stage cardiac injuries.*

***Keywords**: Thallium radioisotopes, doxorubicin, cardiac injury*

INTRODUCTION

Anthracycline chemotherapeutics, including Doxorubicin (DOX), constitute a part of many treatment protocols used to ensure a higher rate of cure in childhood and adulthood malignities (1). With the increase in the cancer rates depending on various factors, such as early diagnosis methods, prolongation of lifetime and carcinogenic substances, the exposure to side effects of these agents have become even more important. Early- and late-onset cardiotoxic side effects are among the causes of significant mortality and morbidity (2).

Endomyocardial biopsy is a method which is considered as the "gold standard" in exhibition of the DOX-induced cardiac injury. Most important disadvantages of the method are its expensiveness and invasiveness (2-4). In addition, myocard diastolic dysfunction, which is one of the earliest findings of the cardiac injury, can be evaluated with Equilibrium Radionuclid angiocardiographic or echocardiographic methods (2). On the other hand, DOX damage is generally irreversible and difficult to predict (2, 5). In order to elucidate the cellular and metabolic changes before the development of the functional disorders, numerous studies have been conducted on a serial of molecular cardiac imaging agents (2). Although the agents like 99mTc-MIBI (methoxyisobutyl isonitrile) and 201Tl (6), which can indicate the myocardial perfusion as well as the cellular integrity successfully revealed the DOX damage, the data concerning the myocard perfusion agents is very limited and uptake mechanisms are not known exactly (7-9).

DOX-induced cardiac injury presents classical findings such as vacuolization in the cytoplasm, myofibrillar degeneration and necrosis under the light microscopy, as well as myocardial inflammation which is another pathological finding that shows inflammatory cell infiltration itself under the light microscopy and is associated with the injury, but can be seen in isolation (4, 10-12). There were various scintigraphic evidences associated with it and support this finding which develops secondary to DOX injury (13-16).

The impact of myocardial inflammation developing secondary to DOX-induced acute cardiac injury on the evolving of scintigraphic findings is not clear (14-16). In the present study, we demonstrated total cardiac T1-201 uptake changes associated with histological findings in early DOX-induced myocardial injury which have not been reported yet.

MATERIAL AND METHODS

Animal treatment and groups

Eighteen adult male albino rats weighing 250 - 350 g were obtained from the "Experimental Animal Care Centre". The study was approved by the "Local Animal Ethics Committee" of the Faculty of Medicine. Animals were divided into three groups (control, acute and subacute phases), each containing six animals. Feed and water were provided ad libitum. DOX treatment groups (both acute and subacute phases) received 15 mg/kg DOX (Adriablastina, 10 mg, Pharmacia Carlo Erba) intraperitoneally. The control group received drinking water without DOX. Cardiac uptake and the other studies has been performed on the 3rd (acute phase) and 16th days (subacute phase) after the treatment.

Cardiac 201Tl uptake study and sample collection

All rats fasted for more than 12 h before the experiments. 201Tl (7.4 MBq; Monrol A.S. Istanbul, Turkey) was injected through the tail vein. The syringe containing the tracer was assessed for radioactivity in a dose calibrator (Atomlab 100 plus dose calibrator, Biodex, NY, USA) before and after the injection for determination of the injected dose in MBq (17). One hour later, intracardiac blood sampling was performed under ketamine–xylazine anesthesia (10–15 and 2–3 mg/kg i.m., respectively). Rats were killed by heart puncture and the hearts removed by dissection. The hearts were cut into two main portion and these samples were carefully weighed. Myocardial uptake of 201Tl was measured with a gama counter (LKB-Wallac 1275 Minigamma counter Wallac, Finland) and calculated as follows (18):

Myocardial uptake (%ID/g x BW) = (myocardial radioactivity / HW) / (total ID / BW)

where ID is injected dose, BW is body weight, and HW is heart weight.

Biochemical assays

Serum was separated by centrifugation at 3000 rpm for 10 min. Lactate dehydrogenase (LDH), creatine kinase (CK) levels were measured kinetically at 340 nm according to the conversion of lactate to pyruvate and the N-acetylcysteine (NAC)-activated reagent methods (19, 20) using orginal Roche diagnostic kits. Analysis of the serum were performed using biochemical analyser (Cobas Integra 800, Roche Diagnostics GmbH, Mannheim, Germany).

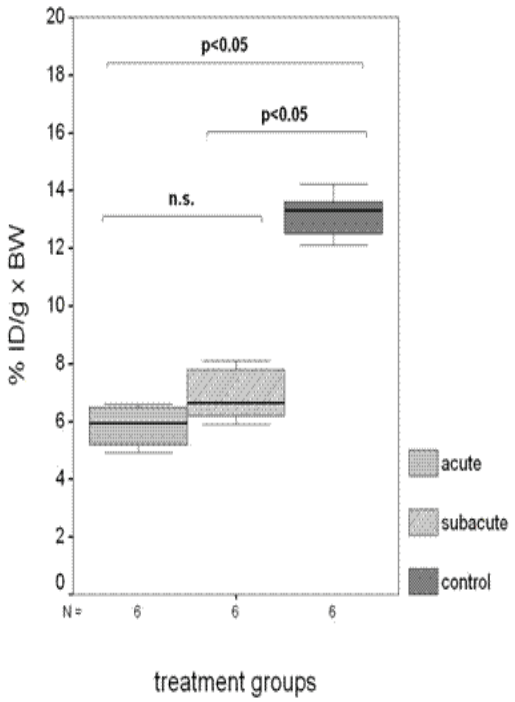

***Figure 1**. Total cardiac uptakes of ^{201}Tl in acute and subacute phases of DOX cardiotoxicity. Medians and interquartile ranges are presented.*

Microscopic Evaluation of Hearts

For histopathological examinations, heart tissue specimens were fixed in 10 % neutral formalin and then embedded in paraffin and cut with a microtome set at a thickness of 5 μM. The sections were stained with hematoxylin-eosin and examined by light microscopy (Olympus BX51, Tokyo, Japan). The right ventricles were examined for typical histopathological features associated with DXR-induced cardiotoxicity. Cardiac scores were determined according to the methods of Saad et al (21, 22). Each specimen was scored for the degree of severity of histopathological changes, (A) Myocardial fiber swelling and interstitial oedema (1+), (B) disorganization of myocardial fiber with or without fibroblastic proliferation (1+), (C) myocardial fiber vacuolation (perinuclear vacuolation) (1+), (D) myocytolysis/necrosis of myocardial fibers (1+), and when no damage was noted (0). Lesion severity in the heart were utilized to calculate a total cardiotoxicity score for each animal.

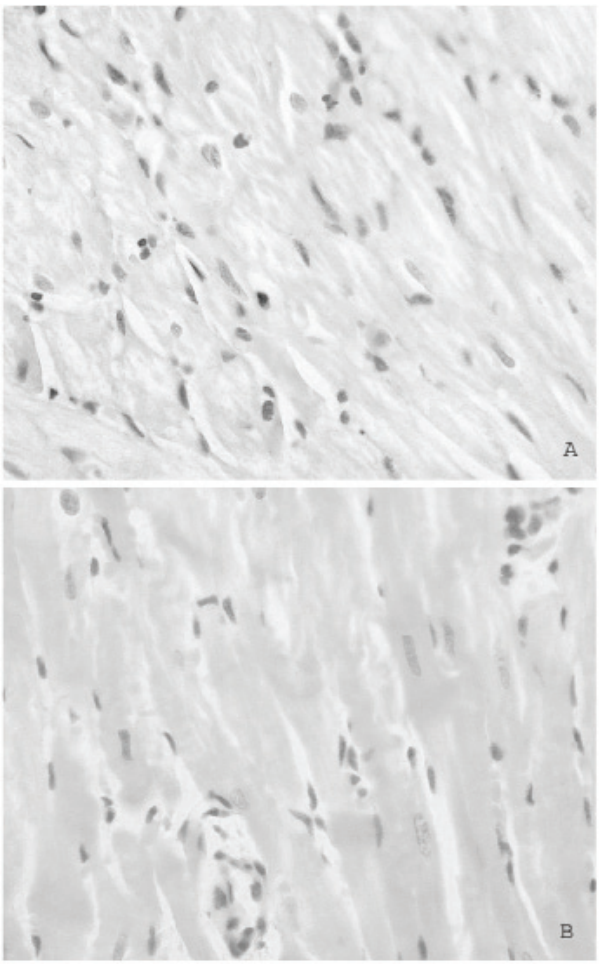

***Figure 2**. Photomicrographs of myocardium section taken from rats treated with DOX depicting marked interstitial oedema, mononuclear cell infiltration, myocardial fiber swelling, and disorganization with perinuclear vacuolation in acute (A) and subacute (B) phases. HEx 400.*

In addition, the specimens were classified using a modification of the previously published criteria (12).

According to the histological degree of mononuclear cell infiltration given as follows, normal (0) (very few mild infiltrating cells in a section, 0–3), mild (1) (a few infiltrating cells in a section, 4-8), moderate (2) (numerous infiltrating cells in a section, 9-13) and severe (3) (numerous infiltrating cells in a section with the area of infiltration, ≥14/mm^2). Lesions and mononuclear cells from 10 random fields (x400) were counted at least two different sections.

Table 1. Serum levels of Creatine Phosphokinase (CK), Lactate Dehydrogenase (LDH) activities of rats treated with DOX.

Group	LDH (IU/ml)	CK (IU/ml)
Control	286 ± 22	334 ± 27
Acute	673 ± 32*	949 ± 44 **
Subacute	983 ± 41*	651 ± 48 **

*Values are the mean ± SD (n=6). Medians marked by the same superscript letters are not significantly different (p > 0.05). * and ** (p = 0.01) vs. control.*

Statistical analysis

Histopathological scores were presented as median and range. Other data were expressed as the mean±SD. Groups parameters were analyzed by using Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by 2-tailed Mann–Whitney's U-test followed by Bonferoni's correction for the paired comparisons. A value of $p<0.05$ was considered to indicate a statistically significant difference. All analysis were performed using SPSS for Windows 11.0.

RESULTS

The percentages of myocardial uptake in the control and DOX treatment groups were shown in Figure 1. ^{201}Tl uptakes as %ID/g x BW were 13.2 ± 0.8; 5.9 ± 0.7; 6.9 ± 0.9 in the control, acute and subacute phases, respectively. DOX led to a significant decrease in myocardial uptake of ^{201}Tl in both the DOX treatment groups ($p<0.05$). There was no significant difference in the %ID/g x BW between acute and subacute phases ($p>0.05$). Enzyme activities were measured in DOX-treated groups in comparison with control. As reported in Table 1, a large increase in the activity of serum LDH and CK which belongs to injury evident was determined in DOX-treated rats ($p=0.01$). There was no significant difference in the LDH and CK level between acute and subacute phases ($p>0.05$).

The histopathological changes in the myocardium are given in Table 2 and Figure 2. DOX treatment produced disorganization of myocardial fibers, vacuolation of the cardiac myocytes and myocardial necrosis ($p=0.01$). Myocardial swelling and interstitial oedema were slightly more apparent in acute phase when compared with subacute phase ($p>0.05$). These cardiomyocyte injuries were accompanied by increased numbers of mononuclear cells ($p<0.05$). Cardiyomyopathy and mononuclear cell infiltration scores were not significantly different between acute and subacute phases ($p>0.05$).

Table 2. Histopathological Changes in the Myocardium of Rats Treated with DOX.

Group	n	Cardiyomyopathy score		Mononuclear cell infiltration score	
		median	range	median	range
Control	6	0		0	
Acute	6	4*	(3-4)	2 *	(1-3)
Subacute	6	3*	(3-4)	2 *	(2-3)

*Values are the mean ± SD (n=6). Medians marked by the same superscript letters are not significantly different (p > 0.05). * and ** (p = 0.01) vs. control.*

DISCUSSION

The treatment of rats with DOX, at a single dose of 15 mg/kg produced significant increases in serum CK and LDH levels in comparison with saline treated controls because of myocardial injury. Moreover, the histopathological examinations obtained in the present study supported this findings. Our results are in accordance with those reported previously (22-24).

There were limited number of experimental studies on scintigraphic agents which were used widely in coronary heart disease diagnosis and viability studies, in respect to the determination of the DOX-induced cardiac injury. In one study, the authors evaluated the kinetics of 99mTc-MIBI in DOX-treated cultured chick heart cells (7) and they found a decreased accumulation of 99mTc-MIBI in the cells. Contrary to this findings, Yürekli et al. (8) found an increase in the cardiac uptake of 99mTc-MIBI in the DOX-induced acute cardiac injury in a in vivo model. In another study, where chronic cardiac injury assesed with ^{201}Tl which has a different kinetic than 99mTc-MIBI and show redistribution (9), it was found that T1-201 uptake after 180 minutes increased with the progression of histological score (9).

The myocardial uptake results of ^{201}Tl, we obtained in the early phase of cardiotoxicity, are inconsistent with the previous findings of chronic DOX-induced cardiotoxicity Miyagawa et al. (9). On the other hand, our findings displayed a ^{201}Tl biodistribution patterns which were similar to those of the autoimmune myocarditis model of Tokita et al. (18). In the study, the total ^{201}Tl uptake in myocarditis was significantly reduced in comparison to that of the controls in the acute phase, but it recovered to the control uptake in the chronic phase. Moreover,

the decrease in ^{201}Tl uptake in myocarditis displayed close association with severity of the inflammation (25). In various clinical studies, it was reported that different types of perfusion defects may develop in the rest ^{201}Tl scintigraphy (26,27). It was also claimed that the changes in the activities of the ion pumps and the ionophoric effect in sarcoplasm may play a role in the pathogenesis of myocarditis (28, 29).

As it is well known, T1-201 acts as a K+ analogue in mammalian cells and it is taken into the cell with Na/K ATPase (9,30,31). DOX affects the potassium level in various cells (32-36). In these studies conducted on kidney cortex and red blood cell invitro cellular cultures (32,33), a decrease was seen in the potassium ion content. In terms of cardiac electrolite level, there were limited data on "K" permeability in the acute single dose changes, intracellular "K" level decreases (34,35) and a moderate increase in chronic DOX injury (36).

Deterioration at cellular electrolyte level, which forms the possible explanation of the alterations of the cardiac biodistribution were placed among the first findings of the injury process (23). In some studies associated with chronic DOX-induced cardiac injury, it was reported that the myocardial electrolyte changes occured before cellular alterations (23,36). It was suggested that these cellular alterations may result from increased permeability of the cell membrane or decreased activity of the energy-dependent ion pump located within the membrane (36). Significant alterations were reported to occur in the activities of the Membrane-associated Ion Pumps, depending on the DOX and its metabolites (37,38). The alterations of the cardiac biodistribution obtained from the present study in the DOX-induced acute injury can be associated with the inhibition of the Na+/K+-ATPase pump in the sarcolemma, that is being specifically one of these pumps (9,38).

Histopathological changes in DOX groups were found similar to those of the literature (22,23,39-42). Extensive vacuolization in the cytoplasm, myofibrillar degeneration and necrosis were among the most typical findings of the classical light microscopy used on acute and chronic cardiac DOX-induced injuries (22, 23,39-42). However, there were also studies reporting that DOX injury presented associated with myocardial inflammation, in addition to producing classical light microscopy findings (4,10,11). Inflammatory infiltration can be detected histologically in high acute doses (21,43) or at chronic toxicity in early stage (22,36,39,44). An increase was reported in the cardiac Indium-111-antimyosin uptake in early period, even before the completion of chemotherapy cures in standard doses in patients who are given DOX treatment (14-16). Considering that the Indium-111-antimyosin was not specific to apoptosis (2,45), it was concluded that the inflammation in the myocard at early stage depending on the DOX can be associated with the occurrence of the sintigraphic results (14-16).

Consequently, we concluded that the DOX-induced cardiac injury at early stage can be evaluated by 201Tl and the findings may be associated with the myocardial inflammation. Due to the complicated mechanism of DOX injury, we strongly believe that the development stage of cardiac injury and the pathological findings should be taken into consideration in interpreting the radiopharmasotic studies to be conducted for the evaluation of the early and late stage cardiac injuries.

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