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The Cardioprotective Effect of N-acetylcysteine on Doxorubicin Induced Cardiotoxicity in Heart Tissue of Rats.

Suat Çakına[1], Latife Ceyda İrkin[2], Şamil Öztürk[3]

[1]Çanakkale Onsekiz Mart University, Health Service Vocational College, Çanakkale-Turkey

[2]Çanakkale Onsekiz Mart University, Canakkale Faculty of Applied Sciences, Department of Fisheries Technology, Çanakkale-Turkey

[3]Çanakkale Onsekiz Mart University, Health Service Vocational College, Çanakkale-Turkey

ABSTRACT: Doxorubicin (DOX) is a chemotherapeutic agent and is widely used in cancer treatment. There are some studies suggesting oxidative stress-induced toxic changes in the liver due to DOX administration. The aim of this study was to reveal the oxidative damage of DOX in liver tissue at molecular level and to evaluate the protective effect of N-acetylcysteine (NAC) against DOX oxidative damage.

Twenty four rats weighing 150-200 g were randomly divided into four equal groups; group I : control, group 2: received a single dose of DOX, group 3: received NAC for 28 days and group 4: received a single dose of DOX, followed by NAC for 28 days. At the end of the experiment, heart tissues were taken from all animals. Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), Total Oxidant Capacity (TOC) levels were determined in these samples by spectrophotometric methods.

It was determined that TOC level increased, TAC levels decreased in the group given DOX compared to the control group. In addition, TAC levels increased in the DOX+NAC group ($p<0.05$).

It was concluded that DOX administration increased oxidative stress and NAC administration could prevent it. NAC caused modulatory effects on oxidative stress and antioxidant redox system in DOX-induced heart toxicity in the rat.

KEYWORDS: Doxorubicin, N-Acetylcysteine, Heart, Oxidative stress.

I. INTRODUCTION

Doxorubicin (DOX) is an antineoplastic anthracycline antibiotic commonly used in the treatment of adult and pediatric cancers such as breast cancer and lymphoma (Elsherbiny, Salama, Said, El-Sherbiny, & Al-Gayyar, 2016; Hajra, Basu, Singha Roy, Patra, & Bhattacharya, 2017; Polegato et al., 2015). Doxorubicin is a toxic agent, it causes toxicity in many organs and tissues in the organism. One of these organs, the heart, is more sensitive to DOX-induced lipid peroxidation and toxicity (Shahira et al., 2016; Singh et al., 2015). The heart also lacks the antioxidant enzymes needed to detoxify oxidants. Therefore, free radicals accumulate and cause lipid peroxidation (Lamas et al., 2015; Migrino et al., 2008).

The molecular mechanism of the cardiotoxic effect of DOX is multifactorial and has not been fully elucidated. The toxic damage produced by DOX is dose-dependent and may ultimately lead to cardiomyopathy. The main reason for DOX-induced cardiotoxicity is the increase in oxidative stress level due to the imbalance in ROS and reactive nitrogen species (RNS) levels. The heart lacks the antioxidant enzymes needed to reduce oxidative stress. Free radicals thus produced accumulate and cause severe lipid peroxidation, resulting in damage to heart tissue (Cassidy, Chan, Rowland, & Allen, 1998; Hrelia et al., 2002; Olson & Mushlin, 1990).

N-acetylcysteine (NAC) is an important antioxidant that plays a role in reducing oxidative stress in the cell. NAC, a precursor of glutathione (GSH), increases intracellular GSH content, stabilizes the cell membrane, maintains cellular viability and directly scavenges ROS (Mansour, El kiki, & Hasan, 2015; Wang et al., 2014). In this study, we aimed to determine the effect of NAC on DOX-induced oxidative stress.

II. RESEARCH METHODS

Adult Wistar Albino rats obtained from Çanakkale Onsekiz Mart University Experimental Research and Application Center were used in this study. Animals had free access to standard rodent food and water under international guidelines standard environmental conditions throughout the study period. Our work was carried out in accordance with the guidelines of the Ethical Committee of Çanakkale Onsekiz Mart University Faculty of Medicine approved the current study (Ethics number: 2021-2100048054). Experiments were conducted in accordance with international guidelines for the ethical use of rats.

Rats were randomly divided in to four experimental groups (6 rats per group) as follows:

- Group 1: Control
- Group 2: DOX (20 mg/kg, intraperitoneal)
- Group 3: NAC (50 mg/kg/day, via gavage) for 28 days
- Group 4: DOX (20 mg/kg Doksorubisin, intraperitoneal) + NAC (50 mg/kg/day, via gavage) for 28 days (from the day of DOX administration)

At the end of the experimental period, the rats were anesthetized by ketamine and xylazine hydrochloride. Then, heart was moved for measuring the oxidative stress markers the rats.

Tissue samples were homogenized in phosphate buffer solution (1:10 w/v, pH: 7.4) using ice-cooled tubes. The homogenate was centrifuged (14,000 rpm, 30 min) and the supernatants were separated for analysis. Protein concentration was estimated by the method of Lowry 10. Tissue samples, taken for malondialdehyde (MDA) determination, were homogenized and subjected to procedures as outlined before (Ohkawa, Ohishi, & Yagi, 1979). TAC and TOC levels were measured by a spectrophotometric assay using commercially available kits (Rel Assay Diagnostics, Turkey). The OSI was defined as the ratio of the TAC level to TOC level.

Statistical analysis: Statistical software (IBM SPSS version 19.0, USA) was used to analyze the data obtained. Comparison of multiple groups was determined by analysis of variance (one-way ANOVA) with post hoc Duncan test. Differences were considered significant at $P<0.05$. All variables were represented as mean ± standard error of the mean (SE).

III. RESULTS

MDA analysis results

In group 2 given DOX, MDA levels increased compared to the control group ($p<0.001$). In group 4 given DOX+NAC; MDA levels decreased compared to the DOX group and control group ($p<0.001$). (Table 1.)

Table 1. MDAanalysis results

Group	MDA (nmole /g)	P Value
1.Control	34.73±1.38	
2.DOX	38.67±2.12	§p:<0.001
3.NAC	29.39±0.48	
4.DOX+NAC	29.88±0.24	‡p , ♯p:<0.001
Group Comparisons: §p= 1 vs. 2; ¶p= 1 vs. 3; ‡p= 1 vs. 4; ♯p: 2 vs. 4		

TAC analysis results

In group 4 given DOX+NAC; TAC levels increased compared to the DOX group ($p<0.001$). In group 2 given DOX, TAC levels decreased compared to the control group ($p<0.001$). (Table 2.)

Table 2. TAC analysis results

Group	TAC (mmole /L)	P Value
1.Control	1.30±0.25	
2.DOX	0.77±0.01	§p:<0.001
3.NAC	1.09±0.25	
4.DOX+NAC	1.22±0.19	♯p:<0.001
Group Comparisons: §p= 1 vs. 2; ¶p= 1 vs. 3; ‡p= 1 vs. 4; ♯p: 2 vs. 4		

TOC analysis results

In group 2 given DOX, TOC levels increased compared to the control group (p=0.008). (Table 3.)

Table 3. TOC analysis results

Group	TOC (µmole/L)	P Value
1.Control	1.76±0.39	
2.DOX	2.49±0.37	§p:0.008
3.NAC	2.18±0.37	
4.DOX+NAC	2.14±0.64	

Group Comparisons: §p= 1 vs. 2; ¶p= 1 vs. 3; ‡p= 1 vs. 4; ♯p: 2 vs. 4

OSI analysis results

In group 2 given DOX, OSI ratio decreased compared to the control group. In group 4 given DOX+NAC, OSI ratio increased compared to the group 2 given DOX. (Table 4.) However, no statistical significance was observed between the groups.

Table 4. OSI analysis results

Group	OSI ratio	P Value
1.Control	0.24±0.08	
2.DOX	0.19±0.04	
3.NAC	0.34±0.01	
4.DOX+NAC	0.17±0.05	

OSI = ((TOC, µmole H_2O_2 Equiv./gram protein)/(TAC, µmole H_2O_2 Equiv./gram protein) × 100. Group Comparisons: §p= 1 vs. 2; ¶p= 1 vs. 3; ‡p= 1 vs. 4; ♯p: 2 vs. 4

IV. DISCUSSION

DOX-induced oxidative stress in heart tissue can be defined as an increase in ROS and reactive nitrogen species (RNS) levels. In this case, the decrease in antioxidant levels and the subsequent deterioration of the cell structure are considered to be the cause of DOX-related cardiotoxicity (Hrelia et al., 2002; Olson & Mushlin, 1990).

Studies have reported that various antioxidant agents such as vitamin E, vitamin C, selenium, erdosteine and carvedilol prevent DOX-dependent cardiotoxicity (Boucher et al., 1995; Fadillioğlu, Erdoğan, Söğüt, & Kuku, 2003; Wahab, Akoul, & Abdel-Aziz, 2000; Woźniak & Anuszewska, 2002). Previous animal studies have reported that NAC has an antioxidant effect against tissue damage caused by free radicals in tissues such as the heart, liver and kidney (Fadillioğlu et al., 2003; Kaya et al., 2008; Sathish, Paramasivan, Palani, & Sivanesan, 2011; Sehirli, Sener, Satiroglu, & Ayanoğlu-Dülger, 2003). We chose NAC in our study because it is a strong antioxidant. Arıcı et. al. reported that heart tissue MDA level was highest in the DOC group. This finding supports the hypothesis that DOX toxicity is primarily due to free radical injury and NAC seems to prevent this damage (Arica et al., 2013).

Short-term findings show that MDA levels in the DOX group were significantly higher than in the DOX + NAC group ($p < 0.05$). Additionally, TAC levels increased significantly and TOC and OSI levels decreased significantly in the DOX+NAC group compared to the DOX group. Our results were consistent with the thesis that DOX-induced heart damage and structural changes could be reduced by the application of an antioxidant. Our results show that NAC application prevents tissue damage against DOX-dependent cardiotoxicity.

V. CONCLUSIONS

In conclusion, NAC was shown to be effective as a biochemical agent in preventing DOX-induced cardiotoxicity in rat models. More clinical trials on cancer patients are needed to evaluate the effect of NAC on DOX toxicity, how NAC affects the antitumor properties of DOX.

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