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Melatonin increased vitamin C and antioxidant enzyme values in the plasma, heart, liver, and kidney of Adriamycin-treated rats

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Abstract: Adriamycin, an anticarcinogenic agent, causes excessive production of reactive oxygen species (ROS). These radicals may affect antioxidant defense systems. The present study was designed to investigate the effects of melatonin on antioxidant enzyme activities and vitamin C in Adriamycin-treated rats. In the current study, rats were divided into three groups: control, Adriamycin, and melatonin+Adriamycin groups. The control group was intraperitoneally injected physiological saline (0.9%) as a placebo. The Adriamycin and melatonin+Adriamycin groups were administered Adriamycin (25 mg/kg body weight). The melatonin+Adriamycin group was pretreated with melatonin (0.5 mg/kg body weight). Vitamin C concentration and catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and myeloperoxidase (MPO) activities were determined in the plasma, heart, liver, and kidneys in the Adriamycin-treated rats. The activities of CAT, SOD, GSH-Px, GR, and MPO decreased generally (between $P < 0.045$ and $P < 0.006$) with Adriamycin treatment, but these enzymes were near to the control values with melatonin treatment. In conclusion, melatonin inhibited Adriamycin-induced ROS production through supporting antioxidant enzyme systems in the plasma, heart, liver, and kidneys of rats, and thus may play an important role against Adriamycin toxicity.

Key words: Adriamycin, melatonin, antioxidant enzymes, heart, liver, kidney, rat

1. Introduction

Adriamycin is an important chemotherapeutic agent used for treatment of some cancer varieties, but it has seriously toxic effects in especially in the heart (Koçkar et al., 2010; Uguz et al., 2012; Zang et al., 2013; Bilginoglu et al., 2014), liver (Aydoğan et al., 2013; Lee et al., 2013), kidney (Hrenak et al., 2013; Escribano et al., 2014), and other tissues (Agopito et al., 2001). However, the mechanism of Adriamycin toxicity is not completely understood. Several mechanisms have been suggested to explain Adriamycin cytotoxicity (Gewirtz et al., 1999), including stabilization of DNA-topomerase complex (Guano et al., 1999), intercalation into DNA (Kiyomiya et al., 2001), and increasing of reactive oxygen species and semiquinone radicals induced by Adriamycin (Kalyanaraman et al., 2002; Othman et al., 2008). Adriamycin is transformed to semiquinone free radicals by the NADPH-cytochrome P450 microsomal system. NADPH-dependent reductase converts Adriamycin to semiquinone free reactive radicals (Vora et al., 1996). Increasing of these radicals during biotransformation of Adriamycin may play a major role in Adriamycin-induced toxicity (Monti et al., 1996; Vora et al., 1996), causing oxidative stress of cellular components in cardiac (Kim et al., 2005; Özdoğan et al., 2011; Zang et al., 2013), hepatic (Aydoğan et al., 2013; Lee et al., 2013), and renal tissues (Hrenak et al., 2013; Escribano et al., 2014).

Oxidative stress is characterized as an imbalance between antioxidant defense systems and reactive oxygen species and nitrogen reactive substances (Nazıroğlu, 2007, 2015). One practice used for minimizing the oxidative stress induced by Adriamycin is the administration of some antioxidants to experimental animals. Melatonin, an important natural antioxidant, may reduce oxidative stress induced by Adriamycin (Othman et al., 2008) and other stimulative agents (Naziroğlu et al., 2012, Senol et al., 2014). It has been reported that Adriamycin affected the activities of CAT, SOD, GSH-Px, and GR in experimental animals (Özdoğan et al., 2011; Lee et al., 2013; Zang et al., 2013). There are many reports documenting protective effects of melatonin on experimental Adriamycin toxicity (Othman et al., 2008; Lee et al., 2013). Melatonin may diminish the cytotoxic effects of the intermediary reactive metabolites produced during biotransformation of Adriamycin in the

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cytochrome P450 microsomal system. Thus, melatonin can scavenge efficiently free radicals before they initiate oxidative damage in cellular components and contributes to physiological functions of the antioxidant defensive system. Therefore, the present study was carried out to investigate the probable effects of melatonin on vitamin C concentrations and CAT, SOD, GSH-Px, GR, and MPO activities in the cardiac, hepatic, and renal tissues and plasma of rats experimentally treated with Adriamycin.

2. Materials and methods

2.1. Animals and treatments

This study was conducted with rats (Wistar albino) aged 2–2.5 months that weighed 150–200 g. All the rats were fed rodent pellets and water ad libitum, and were housed in cages at room temperature with a light/day cycle. Animal housing and the experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*. All rats (n = 24) were randomly divided into three groups. The first group (n = 8) was the control group and physiological saline (0.9%) was intraperitoneal injected into these animals as a placebo. The second group (n = 8) was the Adriamycin group and only Adriamycin was injected (25 mg/kg body weight). The third group (n = 8) received intraperitoneally injected Adriamycin (25 mg/kg body weight) and melatonin (0.5 mg/kg body weight). All chemicals were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck Chemical Co. (Darmstadt, Germany). The Adriamycin and melatonin treatments were applied every other day during the study. After 24 h of the last dose being administered on day 10, blood samples were taken under ether sedation.

2.2. Preparation of plasma and tissue samples

Twenty-four hours after the last dose was administered, blood samples were taken by cardiac puncture under ether sedation; then the animals were sacrificed under ether anesthesia. Blood samples were obtained via cardiac puncture 24 h after the last application of Adriamycin and melatonin. Whole blood was collected into heparinized tubes (Becton Dickinson Vacutainer System, France) and subsequently centrifuged at 1500 × *g* for 15 min in a Heraeus Megafuge 10. Their plasma samples were placed in disposable pipettes and stored at –80 °C for further biochemical analysis. The abdomens of all rats were opened via laparotomy and their livers, hearts, and kidneys were removed. These organs were then rinsed quickly in cold saline solution and divided into two halves. These tissues were homogenized and they were frozen at –80 °C until the antioxidant enzymes assays. Then the enzyme activities and vitamin C concentrations in the plasma, heart, liver, and kidneys samples were determined.

2.3. Biochemical analyses

The abdomens of all rats were opened and their hearts, liver, and kidneys were removed. These tissues were stored at –80 °C for further biochemical analysis. All the analyses were conducted as suggested by the manufacturers of commercial kits. Catalase (CAT) activity was assayed according to Goth (1991). Myeloperoxidase activity in the tissue specimens was analyzed according to Krawisz et al. (1984). Total SOD activity was determined as reported by Sun et al. (1988). GSH-Px activity was measured using the method described by Paglia and Valentine (1967), while GR activity was measured using the method described by Carlberg et al. (1986). Protein contents were measured according to the method of Lowry et al. (1951). Vitamin C concentrations were measured according to the method reported by Omaye et al. (1979). All antioxidant enzymes were analyzed by spectrophotometry (Jenway 6800 UV-1175, China).

2.4. Statistical analysis

Statistical analysis was performed using SPSS v.11.5 (SPSS, Inc., Chicago, IL, USA). The data were expressed as means ± standard deviation (SD). Differences between group means were estimated using one way analysis of variance followed by Mann–Whitney U-test and the results were considered to be statistically significant at $P < 0.05$.

3. Results

The activities of CAT, SOD, GSH-Px, GR, and MPO enzymes in the plasma, heart, liver, and kidneys are presented in Tables 1–4. Vitamin C concentrations in the plasma, heart, liver, and kidneys are shown in Figures 1A–1D.

The activities of CAT, SOD, GSH-Px, GSH-Rx, and MPO and vitamin C in plasma decreased significantly with Adriamycin treatment, while CAT, SOD, and MPO activities and vitamin C concentration increased significantly (Table 1; Figure 1A). GSH-Px and GSH-Rx activities were not affected (almost the level observed in the control group) by Adriamycin plus melatonin treatment.

In cardiac tissue, while CAT and SOD activities decreased significantly, GSH-Px, GR, and MPO activities increased in response to Adriamycin. In addition, SOD increased, MPO decreased significantly, and CAT, GSH-Px, GR and vitamin C were not affected by Adriamycin plus melatonin treatment (Table 2).

In hepatic tissue, while CAT, GSH-Px, and MPO activities increased significantly in response to Adriamycin, SOD and GR were decreased significantly by Adriamycin. In addition, while the activities of CAT, GSH-Px, GR, and MPO decreased, the values of SOD significantly increased in response to Adriamycin plus melatonin treatment (Table 3).

Table 1. Effects of melatonin on catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and myeloperoxidase (MPO) activities in plasma of Adriamycin-treated rats (n = 8).

Parameters/Groups	Control	Adriamycin	Adriamycin+melatonin
CAT, U/L	649.04± 103.63	487.28±113.26[a]	693.27± 16.40[f]
SOD, U/L	73.44± 9.32	56.26± 7.41[b]	73.47± 6.02[g]
GSH-Px, U/L	398.98± 69.06	227.46± 125.25[c]	235.47± 123.43[a]
GR, U/L	42.14± 6.16	29.84± 6.46[c]	26.30± 3.71[e]
MPO, U/L	73.84± 19.47	37.84± 9.82[d]	68.83± 25.61[h]

Data are presented as mean ± SD.
Statistical significance versus control group, [a]P < 0.046, [b]P < 0.036, [c]P < 0.028, [d]P < 0.016.
Statistical significance versus Adriamycin group, [e]P < 0.049, [f]P < 0.006, [g]p < 0.011, [h]P < 0.052.

Table 2. Effects of melatonin on catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and myeloperoxidase (MPO) activities in cardiac tissue of Adriamycin-treated rats (n = 8).

Parameters/Groups	Control	Adriamycin	Adriamycin+melatonin
CAT, U/g protein	548.32± 41.86	398.80± 49.96[b]	319.27± 147.37[c]
SOD, U/g protein	6.37± 0.68	5.73± 0.53[a]	7.16± 0.25[b,e]
GSH-Px, U/g protein	122.97± 7.93	157.42± 16.18[b]	147.42± 18.95[a]
GR, U/g protein	39.97± 12.32	50.26± 6.83[b]	50.84± 7.62[c]
MPO, U/g protein	8.02± 1.20	15.65± 0.67[b]	9.23± 2.36[e]

Data are presented as mean ± SD.
Statistical significance versus control group, [a]P < 0.046, [b]P < 0.009, [c]P < 0.006.
Statistical significance versus Adriamycin group, [d]P < 0.047, [e]P < 0.006.

Table 3. Effects of melatonin on catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and myeloperoxidase (MPO) activities in hepatic tissue of Adriamycin-treated rats (n = 8).

Parameters/Groups	Control	Adriamycin	Adriamycin+melatonin
CAT, U/g protein	4.24± 2.90	9.92± 4.13[b]	67.72± 33.93[b,g]
SOD U/g protein	5.12± 0.29	4.67± 0.56[a]	5.57± 0.25[a,f]
GSH-Px, U/g protein	31.18± 18.81	68.59± 19.33[a]	59.99± 21.51[e]
GR, U/g protein	212.88± 9.87	198.12± 16.04[a]	178.63± 16.63[d,e]
MPO, U/g protein	6.44± 0.84	11.51± 3.03[c]	7.25± 2.55[d,f]

Data are presented as mean ± SD.
Statistical significance versus control group, [a]P < 0.046, [b]P < 0.027, [c]P < 0.009, [d]P < 0.006.
Statistical significance versus Adriamycin group, [e]P < 0.045, [f]P < 0.017, [g]P < 0.009.

Table 4. Effects of melatonin on catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and myeloperoxidase (MPO) activities in renal tissue of Adriamycin-treated rats (n = 8).

Parameters/Groups	Control	Adriamycin	Adriamycin+melatonin
CAT, U/g protein	95.80± 13.92	10.76± 4.03	109.90± 17.83[e]
SOD, U/g protein	5.94± 0.41	6.30± 0.81	6.79± 0.61
GSH-Px, U/g protein	154.45± 31.84	85.80± 8.75[b]	139.90± 13.69[e]
GR, U/g protein	215.98± 67.85	222.87± 21.65	212.74± 80.41
MPO, U/g protein	5.42± 1.45	13.22± 3.71[c]	6.67± 1.38[e]

Data are presented as mean ± SD.
Statistical significance versus control group, [a]P < 0.045, [b]P < 0.016, [c]P < 0.009.
Statistical significance versus Adriamycin group, [d]P < 0.044, [e]P < 0.006.

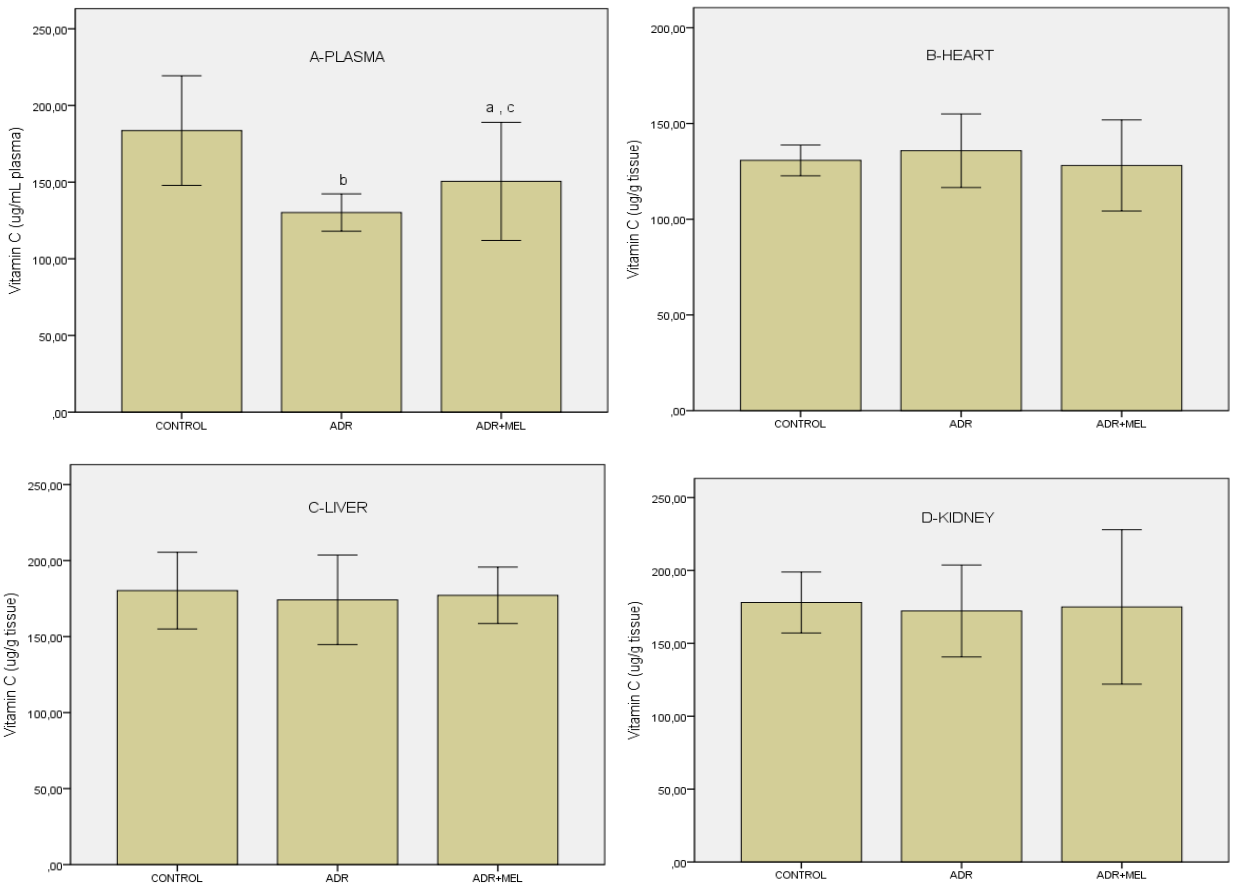

Figure 1. Vitamin C concentrations in (A) plasma, (B) heart, (C) liver, and (D) kidney in all groups.
Data are presented as mean ± SD.
CONT: Control; ADR: Adriamycin; MEL: Melatonin.
Statistical significance versus control group, [a]P < 0.018, [b]P < 0.010.
Statistical significance versus Adriamycin group, [c]P < 0.044.

In renal tissue, while GSH-Px activity decreased significantly, MPO activity increased, but CAT, SOD, and GSH-Rx activities were not affected by Adriamycin. Moreover, while GSH-Px was increased significantly, MPO was decreased significantly by Adriamycin plus melatonin treatment (Table 4).

4. Discussion

Several authors have confirmed the protective effect of melatonin on different tissues during Adriamycin intoxication (Agopito et al., 2001; Zang et al., 2013; Bilginoglu et al., 2014). Adriamycin is highly toxic to the heart, liver, kidney, and small intestine. Studies have suggested that melatonin scavenged the highly toxic hydroxyl radicals induced by Adriamycin (Tan et al., 1998; Agopito et al., 2001; Othman et al., 2008) and other stimulants (Naziroğlu et al., 2012, Senol et al., 2014). Furthermore, Adriamycin is transformed to semiquinone free radicals by the NADPH-cytochrome P450 microsomal system. NADPH-dependent reductase converts Adriamycin to semiquinone free radicals, which then leads to the generation of superoxide anion and hydroxyl radicals damaged to cellular lipids and lipoproteins. These radicals may play an important role in Adriamycin-induced cytotoxicity (Vora et al., 1996; Agopito et al., 2001; Lee et al., 2013). Thus, Adriamycin-induced toxicity may contribute to a reduction in the antioxidant defense system in the heart and other tissues due to oxidative damage from these free radicals (Özdoğan et al., 2011).

It has been reported that the values of SOD and GSH-Px may be important indicators in myocardial injury related to the degree of Adriamycin toxicity (Floyd et al., 2005). In the present study, plasma activities of CAT, SOD, GSH-Px, and GR were decreased significantly in response to Adriamycin (Table 1). In addition, MPO activity decreased in plasma, but cardiac, hepatic, and renal MPO activities were increased significantly in response to Adriamycin. In addition, hepatic CAT and GSH-Px activities increased, and SOD and GR activities decreased significantly in response to Adriamycin (Tables 2–4). These results are consistent with those of studies reporting increased activities of MPO (El Berry et al., 2010) and CAT, SOD, and GSH-Px with Adriamycin induction (Özdoğan et al., 2011; Lee et al., 2013).

Melatonin, an important hormone of the pineal gland, is produced by various other tissues including the retina (Tosini and Menaker, 1998), gastrointestinal tract (Bubenik, 2002), skin (Slominski et al., 2005), lymphocytes (Carrillo-Vico et al., 2004), and bone marrow (Conti et al., 2000). The antioxidant activity of melatonin has been described to occur by two different mechanisms: (1) melatonin directly scavenges •OH radicals (Kim et al., 2005) and (2) melatonin reduces oxidative stress by stimulating antioxidant enzymes (Reiter et al., 1997). Oxidative stress is characterized as an imbalance between antioxidant defense systems and reactive oxygen species (ROS) generated during many aerobic physiological processes such as mitochondrial electron transfer reactions, phagocytic activity, and nitrogen reactive substances (Nazıroğlu, 2007, 2009).

CAT is an antioxidant defense enzyme and a potent H_2O_2 scavenger. This enzyme may prevent the formation of highly toxic hydroxyl radicals (Yabe et al., 2001; Aydoğan et al., 2013). Thus, the production of CAT provides additional antioxidative activity against oxidative stress during oxidative injury. In the present study, hepatic activities of CAT, GSH-Px, GR, and MPO decreased significantly with melatonin treatment (Table 3). In contrast, CAT activity in the plasma and kidney significantly increased with melatonin treatment (Tables 1 and 4). In the present study, SOD activity increased significantly in the plasma, heart, and liver; renal GSH-Px increased; and renal MPO decreased with melatonin treatment (Table 4). These findings are partially consistent with the results concerning decreases in CAT, SOD, and GSH-Px with melatonin (Özdoğan et al., 2011; Lee et al., 2013). The accumulating evidence implicates a modulatory role of melatonin on excessive oxidative stress induced in Adriamycin toxicity (Othman et al., 2008; Lee et al., 2013; Escribano et al., 2014) and some stimulative agents (Nazıroğlu et al., 2012; Şenol et al., 2014; Nazıroğlu, 2015).

Plasma antioxidants may be more susceptible than tissue antioxidants to Adriamycin toxicity and melatonin treatment. Adriamycin-induced oxidative damage may increase ROS such as superoxide ($O_2.^-$), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and other free oxygen radicals. The mechanism underlying this cytotoxicity seems to be linked to an increased production of ROS and oxidative damage (Berthiaume and Walke, 2007; Özdoğan et al., 2011). In plasma samples, while vitamin C decreased in response to Adriamycin, this antioxidant vitamin was increased significantly by melatonin treatment (Figure 1A). Furthermore, cardiac, hepatic, and renal vitamin C values were not affected by melatonin treatment (Figures 1B–1D). It has been reported that vitamin C decreased oxidative stress (lipid hydroperoxides and total oxidant status and oxidative stress index) induced by acetic acid in rats (Zerin et al., 2010). Vitamin C is a well-known antioxidant that has been shown to efficiently scavenge free oxygen radicals, including superoxide, hydrogen peroxide, hypochlorite, hydroxyl, peroxyl, and singlet oxygen. Thus, vitamin C may diminish certain types of lipid peroxidation and may play a major role in preventing oxidative stress in cardiac, hepatic, and renal tissues of rats (Loo et al., 2003). All these results may provide scientific support in understanding the protective effects of melatonin on plasma antioxidants in oxidative damage produced due to Adriamycin toxicity.

In conclusion, the present study indicated that Adriamycin and melatonin affected antioxidant enzymes and plasma vitamin C concentration. Increasing free oxygen radicals due to peroxidation reactions produced during Adriamycin biotransformation may increase

oxidative damage in the cellular components of living organisms. Thus, melatonin may play an important protective role against Adriamycin toxicity in the plasma, heart, liver, and kidney. This protective role may be due to both decreasing of oxidative stress and induction of some antioxidant enzymes. Chemotherapy with Adriamycin may cause a risk of cardiac, hepatic, and renal damage. Therefore, melatonin may be a therapeutic adjuvant that may modulate oxidative stress induced by Adriamycin toxicity. However, there is a need for more detailed studies in order to assess possible relationships between antioxidants and Adriamycin-induced toxicity. We have been considering the possibility that antioxidant administration (like β-carotene and vitamin E) may have a prophylactic role in modulation of complications in Adriamycin toxicity. Therefore, we are presently continuing our studies investigating the effects of Adriamycin toxicity.

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