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The Protective Effects of Amifostine on Adriamycin-Induced Acute Cardiotoxicity in Rats

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Department of Internal Medicine, Division of Hematology, [1]Division of Oncology, [2]Deparment of Biochemistry, Adnan Menderes University Medical School, Aydin, Turkey, and
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Bolaman, Z., Cicek, C., Kadikoylu, G., Barutca, S., Serter, M., Yenisey, C. and Alper, G. *The Protective Effects of Amifostine on Adriamycin-Induced Acute Cardiotoxicity in Rats*. Tohoku J. Exp. Med., 2005, **207** (4), 249-253 —— Free oxygen radicals and lipid peroxidation are responsible for adriamycin-induced cardiotoxicity. Amifostine is a scavenger of free radicals and may function as a selective cytoprotective agent. The aim of this study was to investigate the effects of amifostine on adriamycin-induced lipid peroxidation and the levels of protective enzymes in the heart. Male Wistar rats were randomly allocated to three groups: pretreated, untreated, and control (n = 10 in each group). Rats were pretreated with an intraperitoneal injection of amifostine (200 mg/kg) 30 min before the injection of adriamycin. The pretreated rats were given an intraperitoneal injection of adriamycin (10 mg/kg) and were sacrificed after 72 h. Likewise, rats received intraperitoneal injection of adriamycin (untreated) or saline (control). The hearts were removed for the analyses of malondialdehyde (MDA), reduced glutathione (GSH) and catalase. MDA levels were increased ($p < 0.005$) in the heart tissues of untreated rats compared to control, while GSH and catalase levels were decreased ($p < 0.05$ and $p < 0.001$, respectively) in untreated animals. In amifostine-preatreated group, MDA levels were lower ($p < 0.01$), and GSH and catalase levels were higher ($p < 0.05$ for both) than the untreated group. GSH levels were even higher in the amifostine-pretreated group compared to control ($p < 0.01$), although catalase levels were significantly lower in the pretreated group ($p < 0.05$). These results indicate that amifostine decreases adriamycin-induced lipid peroxidation and increases the levels of the protective enzymes in the heart tissue. Therefore, amifostine may ameliorate the adriamycin-induced acute cardiotoxicity. ———— adriamycin; amifostine; acute cardiotoxicity; malondialdehyde; catalase

Adriamycin is an anthracycline antitumor agent that has been used in the treatment of some hematological malignancies and solid tumors (Hortobagyl 1997). However, its therapeutic success is limited due to its cardiotoxicity (Lown et al. 1982; Itoh et al. 2004). Adriamycin causes membrane damage and bio-reductive activation leading to the formation of free oxygen radicals

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such as hydrogen peroxide (H_2O_2), superoxide anions, and hydroxyl radicals (Lown et al. 1982; Tewey et al. 1984; Keizer et al. 1990; Dorr et al. 1996). These radicals are toxic for the myocardium. Free oxygen radicals may contribute to the adriamycin-induced cardiotoxicity (Vermeulen and Baldew 1992; Minotti et al. 1999) by causing diverse oxidative damage on critical cellular components and membrane lipids in cellular organelles, such as plasma membrane and mitochondria (Doroshow and Davies 1986; Dorr et al. 1996; Shan et al. 1996). Moreover, the production of free oxygen radicals induces lipid peroxidation and oxidative damage in the heart (Lown et al. 1982; Keizer et al. 1990; Bhanumathi et al. 1994; Dragojevic-Simic et al. 2004).

For the prevention of adriamycin-induced cardiotoxicity, dexrazoxane, N-acetylcysteine, melatonin (a pineal hormone) and some antioxidants, such as vitamin C, vitamin E and alpha-tocopherol, have been used in experimental and clinical studies (Singal and Tong 1988; Dorr 1996; Samelis et al. 1998; Kocak et al. 2003).

Amifostine decreases generation of free oxygen radicals and might have a protective effect on adriamycin toxicity (Singal and Tong 1988; Dorr 1996; Kang et al. 1996; Samelis et al. 1998; Kocak et al. 2003; Kadikoylu et al. 2004). Adriamycin-induced hematological and testicular toxicities, cataract formation, and cisplatin-induced neuro-, nephro- and ototoxicities may be either prevented or decreased by administration of amifostine before cytotoxic chemotherapy in experimental and clinical studies (Spencer et al. 1995; Santini and Giles 1999). In some studies, it was reported that amifostine might also have favorable effects on adriamycin-induced cardiotoxicity by inhibiting lipid peroxidation and the generation of free oxygen radicals (Dorr et al. 1996; Dobric et al. 1998; Bolaman et al. 2001; Dragojevic-Simic et al. 2004). In this study, we investigated the effects of amifostine on the adriamycin-induced cardiotoxicity in the rat heart.

Materials and Methods

Chemicals

Adriamycin (Adriablastina®) was obtained from Pharmacia Carlo Erba/Turkey as a 10 mg/10 ml lyophilized powder. It was reconstituted in 100 ml of 0.9% sodium chloride for injection, and stored at –80°C in plastic vials before use. Amifostine (Ethyol®) was obtained from TR Er-Kim/Turkey as 500 mg/10 ml. Both drugs were dissolved in double distilled water before intraperitoneal (ip) administration.

Animals

Male Wistar rats, weighing 201 ± 32 g (157 - 259 g), were obtained from Experimental Surgery and Research Center of Aegean University. Rats were kept in ventilated rooms at 23 ± 2°C temperatures, 14 h darkness/10 h light and in relative humidity of 60-75%. They were fed on standard feed (Best Feed/Turkey), water ad libitum. The study included three groups (n = 10 in each group): control group (isotonic saline ip), untreated group (adriamycin 10 mg/kg ip), and pretreated group (adriamycin 10 mg/kg ip plus amifostine 200 mg/kg ip). Amifostine was administered 30 min before adriamycin. The rats were sacrificed 72 h after the drug administration and their hearts were removed. The hearts were washed with cold saline solution, placed into Petri dishes and stored at –80°C. In several experimental studies on adriamycin-induced acute cardiotoxicity a single dose of 6-20 mg/kg adriamycin was used and the evaluations were performed in the first week (Dobric et al. 1998; Bolaman et al. 2001; Kocak et al. 2003). So a single dose of 20 mg/kg adriamycin was administered to rats and cardiotoxicity was evaluated after 72 h. Institutional Animal Care and Use Committee and National Institute of Health Guidelines for animals care were followed throughout the study (Pitts 2002). Local Ethical Committee of Adnan Menderes University had approved this study.

Analysis

The tissues were thawed and homogenized with Ultra-Turax in 50 mM phosphate buffer solution. For the evaluation of lipid peroxidation malondialdehyde (MDA) levels were measured by spectrophotometric/colorimetric methods of Ohkawa et al. (1979). Each 0.2 ml of homogenate was mixed with 1.5 ml of 0.67% thiobarbituric acid (Sigma-Aldrich, St.Louis, MO, USA), 1.5 ml of 20% acetic acid (Merck, Darmstadt, Germany) in pH 3.5 and 0.2 ml of 8.1% sodium dodecyl sulphate (Sigma-Aldrich). A set of MDA standard consisted of 1-40 nmol

malonaldehyde bis (Sigma-Aldrich). After coupling, all samples and standards were heated at 100°C for one hour and then cooled on ice. The absorbance of MDA was measured at 532 nm (Shimadzu 160 spectrophotometer, Kyoto) and the concentration values equivalent to standard curve absorbance values on spectrophotometer were identified as nmol/g tissue weight and then were calculated as nmol/g weight. Glutathione (GSH) levels were measured by using the spectrophotometric/colorimetric method of Tietze (1969). Precipitant, phosphate, and dithio-bis-nitrobenzoic acid solutions were used. Precipitant solution was prepared by using glacial metaphosphoric acid (Seelze, Riedel de Haen, Germany), disodium EDTA (Sigma-Aldrich), and sodium chloride (J.T.Baker, Phillipsburg, NJ, USA). Phosphate solution was prepared by using disodium hydrogen phosphate (Merck). Dithio-bis-nitrobenzoic acid solution was prepared by mixing sodium citrate (Sigma-Aldrich), and dithio-bis-nitrobenzoic acid (Sigma-Aldrich). GSH standards were constituted as 1-60 mg/100 ml by using GSH form. Samples and standards versus blind were read at 412 nm. The results were obtained as μg/mg protein by Lowry's method (Lowry et al. 1985). Catalase activity was measured by the spectrophotometric/enzymatic method of Aebi (1984). Tampon solution was prepared with potassium dihydrogen phosphate (Merck) and disodium hydrogen phosphate (Merck). The samples were diluted with tampon solution and the absorbance after addition of hydrogen peroxide tampon (Prolabo, Paris, France) was recorded at 240 nm at the 15th second. The results were given as U/g tissue weight.

Statistical analysis

All results were given as mean ± S.D. One-way ANOVA and post-hoc tests were used for the comparisons of study groups' results. P values < 0.05 were accepted as statistically significant.

Results

Administration of adriamycin significantly increased the MDA levels ($p < 0.005$) and decreased the levels of GSH and catalase ($p < 0.05$ and $p < 0.001$, respectively) in the heart tissues of the untreated rats, compared to the control (Table 1). There was no significant difference in the heart weights between the groups. In the amifostine-pretreated group, MDA levels were significantly lower than the untreated group ($p < 0.01$), but were still significantly higher than the control ($p < 0.01$). Furthermore, both GSH and catalase levels were significantly higher in the amifostine-pretreated group than the untreated group ($p < 0.05$ for both). It is noteworthy that GSH levels are higher in the pretreated group than the control ($p < 0.01$), whereas catalase activity was significantly lower ($p < 0.05$).

Discussion

The present study has shown that adriamycin induces lipid peroxidation and decreases the levels of protective enzymes in the heart tissues. Amifostine pretreatment significantly reduced the lipid peroxidation and increased the levels of GSH and catalase. These results may indicate

Table 1. *The biochemical values in the heart tissues of the study groups*

	MDA (nmol/g tissue weight)	GSH (μg/mg protein)	Catalase (U/g tissue weight)	Heart weight (g)
Control (n = 10)	274.0 ± 115.3	125 ± 30	3.9 ± 0.63	0.335 ± 0.174
Untreated (n = 10)	887.4 ± 422.9	87 ± 47	2.3 ± 0.80	0.411 ± 0.161
Pretreated (n = 10)	443.6 ± 188.2	161 ± 50	3.1 ± 0.68	0.328 ± 0.119

MDA (malondialdehyde): Untreated vs control group $p < 0.05$; pretreated vs untreated group $p < 0.01$; pretreated vs control group $p < 0.01$.

GSH (glutathione): Untreated vs control group $p < 0.005$; pretreated vs untreated group $p < 0.05$; pretreated vs control group $p < 0.01$.

Catalase: Untreated vs control group $p < 0.001$; pretreated vs untreated group $p < 0.05$; pretreated vs control group $p < 0.05$.

Heart weight: $p > 0.05$.

protective effects of amifostine on adriamycin-induced acute cardiotoxicity.

Adriamycin-induced cardiotoxicity is related to cumulative dosage. The generation of free oxygen radicals and interference with mitochondrial electron transport chain are the accepted mechanisms. Mitochondria are abundant in the heart, and the heart has a tendency to receive the free radical-induced damage due to its dependence on the glutathione-glutathione peroxidase cycle (Steinherz et al. 1991; Shan et al. 1996). Free oxygen radicals can react with unsaturated lipids, leading to lipid peroxidation. These radicals may induce DNA damage (Keizer et al. 1990; Bhanumathi et al. 1994; Dobric et al. 1998).

Amifostine disarms free oxygen radicals on essential molecules, and forms mixed disulfides that protect normal cells. The recommended dose of amifostine is 740-910 mg/m^2 in humans for myelo- and nephroprotection. Pretreatment with amifostine (50-300 mg/kg) had a significant protective effect against various adriamycin-induced toxicities in rats (Holford 1996; Budd et al. 1997; Jahnukainen et al. 2001; Barutca et al. 2004). It has a dose-related protective effect against cardiotoxicity (Dorr 1996; Dobric et al. 1998; Nazeyrollas et al. 1999; Bolaman et al. 2001).

Dexrazoxane, which chelates with intracellular iron and prevents the production of free oxygen radicals in cardiac muscle, is an effective selective protector of adriamycin-induced cardiotoxicity (Swain et al. 1997; Seymour et al. 1998). Studies comparing the preventive effects of amifostine and dexrazoxane on adriamycin-induced cardiotoxicity are very rare. Herman et al. (2000) compared the protective effects of amifostine and dexrazoxane on adriamycin-induced cardiotoxicity. Although dexrazoxane was more cardioprotective than amifostine, and prevented the mortality induced by doxorubicin, mortality was not decreased by amifostine pretreatment. Perhaps a study on the efficacy of amifostine plus dexrazoxane combination for the prevention of adriamycin-induced cardiotoxicity may be designed.

Adriamycin causes depletion of cardiac GSH (Dorr et al. 1996; Zhou et al. 2001). This may reflect the consistent formation of free oxygen radicals (Zhou et al. 2001). In the present study, adriamycin significantly decreased the GSH levels, but the GSH levels were significantly higher in the amifostine-pretreated group than controls. It has been reported that amifostine increased GSH levels in rats (Issels and Nagele 1989; Dorr et al. 1996; Stankiewicz et al. 2002). So, amifostine may have favorable effects on adriamycin-induced acute cardiotoxicity.

Catalase is another antioxidant enzyme in the cellular defense system against free oxygen radicals (Tewey et al. 1984). In our study, adriamycin decreased the catalase activity in the heart tissues of untreated and amifostine-pretreated groups. Although the difference was not significant, the degree of decrement was smaller in the amifostine-pretreated group.

In conclusion, adriamycin increased lipid peroxidation and reduced the levels of GSH and catalase in rat heart, whereas the pretreatment with amifostine decreased lipid peroxidation and increased the protective enzymes. These results suggest that amifostine may ameliorate the adriamycin-induced acute cardiotoxicity.

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