Full Length Research Paper

Antioxidant defense of glutamine on myocardial antioxidant status in adriamycin-induced cardiomyopathy in rats

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Oxidative stress is one of the major factors involved in the pathogenesis of adriamycin-induced cardiac dysfunction. The present study examined the antioxidant defense of glutamine on myocardial antioxidant status in adriamycin-induced cardiomyopathy in rats with respect to changes in the levels of lipid peroxidation and reduced glutathione, and the activities of antiperoxidative enzymes [superoxide dismutase (SOD) and catalase (CAT)] and glutathione dependent antioxidant enzymes [glutathione peroxidase (GPx) and glutathione-S-transferase (GST)] in the heart tissue. Intraperitoneal injection of adriamycin caused a significant (P<0.001) rise in the level of lipid peroxidation in the heart tissue with a concomitant (P<0.001) decline in the level of reduced glutathione and the activities of the antioxidant enzymes. Oral administration of glutamine significantly (P<0.001) ameliorated these adriamycin induced adverse effects and maintained the level of the evaluated parameters nearly at normal. The results of the present investigation demonstrated that the protective effects of glutamine against the toxicity of the prooxidant antitumor drug, adriamycin, might be attributed at least partially to its antioxidant properties.

Key words: Glutamine, adriamycin, cardiomyopathy, lipid peroxidation, reduced glutathione, antioxidant enzymes.

INTRODUCTION

Adriamycin is one of the most effective and widely used chemotherapeutic agents against leukemia, lymphomas and various solid tumors of the lung, breast, thyroid and ovary (Kim et al., 2005). The congestive heart failure induced by adriamycin is proven refractory to commonly used therapeutic procedures (Rajaprabhu et al., 2007). It induces cardiomyopathy by a multiple step mechanism. Peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of adriamycin (Chularojmontri et al., 2005). Adriamycin-induced oxidative stress is generally attributed to the formation of the highly reactive hydroxyl radical (OH•), stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Zhou et al., 1999). The major abnor-

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transamination via α-ketoglutarate and glutamate. It provides nitrogen for a number of biosynthetic pathways, serving as a precursor of the purine and pyrimidine rings of nucleic and nucleotides such as adenosine triphosphate (ATP) (Windmueller and Spaeth, 1974). Glutamine plays an important role in the nitrogen and carbon-skeleton exchange among different tissues, where this amino acid fulfills many different physiological functions (Kovaccvic and McGivan, 1983). It has immunoregulatory and cell-regulative capabilities, as recent investigations have shown (Roth et al., 2002). It also regulates endothelial nitric oxide metabolism in the heart tissue (Murphy and Newsholme, 1997). It is also involved in cell membrane stabilization, antioxidation, detoxification, and energy production (Rennie et al., 1994; Matilla et al., 2000; Fox et al., 1996). Reduction in the level of intracellular concentration of glutamine has been reported to occur in the heart tissue during myocardial dysfunction (Suleiman et al., 1993). Though glutamine is assumed to participate in various important biological and physiological functions in heart, the antioxidant defense of glutamine on myocardial antioxidant defense system in experimentally induced cardiomyopathy condition has not been explored in detail. In the present study, we have attempted to assess the cardioprotective effect of glutamine against adriamycin-induced cardiomyopathy in male albino rats by virtue of its antioxidant, hypolipidemic and membrane stabilizing properties.
Chemicals
Adriamycin, epinephrine, glutamine and tetramethoxy propane were obtained from M/s Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals used were of analytical grade.

Animals
Wistar strain male albino rats, weighing 150 – 180 g, were housed individually in polypropylene cages under hygienic and standard environmental conditions (28±2ºC, humidity 60 - 70%, 12 h light/dark cycle). The animals were allowed a standard diet [M/s Sai Feeds, Bangalore, India] and water ad libitum. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental protocol
The animals were divided into four groups, comprising six rats each. Rats in Group I (normal control) received standard diet and intragastrically administered with distilled water for a period of 15 days. Group II animals were orally administered with glutamine [150mg (dissolved in distilled water)/kg body weight/day] by intragastric intubation for a period of 15 days. In Group III, animals were administered with distilled water for a period of 15 days and also injected with adriamycin [1.5 mg (dissolved in physiological saline)/kg body weight/day, i.p. for 15 days] for the induction of cardiomyopathy (Rajaprabhu et al., 2007). In Group IV, the animals were administered with glutamine [150 mg (dissolved in distilled water)/kg body weight/day] and also injected with adriamycin, as described in Group III for a period of 15 days.

At the end of the experimental period, that is, 24 h after last injection of adriamycin, the animals were killed; the heart tissue was excised immediately and washed with chilled isotonic saline. The heart tissue homogenates prepared in ice cold 0.1M Tris–HCl buffer, pH 7.2, were used for the determination of lipid peroxides (Ohkawa et al., 1979), reduced glutathione (GSH) (Ellman, 1959), glutathione peroxidase (GPx) (Pagila and Valentaine, 1967), glutathione-S-transferase (GST) (Habig et al., 1974), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and catalase (CAT) (Takahara et al., 1960).

### Materials and Methods

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### Results and Discussion

Adriamycin is a quinone-containing anticancer drug that is extensively used to treat a variety of human neoplastic diseases as well as an ample range of solid tumors, including breast, lung and thyroid cancer (Gianni et al., 2007). The clinical efficacy of this drug is greatly restricted on account of it causing a severe form of cardiomyopathy or congestive heart failure in some adriamycin-treated cancer patients. Oxidative stress is known to play an important role in the pathogenesis of adriamycin-induced myocardial injury (Subashini et al., 2006). The focus of the current study was to investigate the effects of glutamine for its antioxidant and membrane-stabilizing properties during adriamycin-induced cardiomyopathy.

Oxidative stress is the result of excessive production of oxidant species and/or depletion of intracellular antioxidant defenses, leading to an imbalance in the redox status of the myocardial cells. Myocardium is a potential target of injury by oxygen radicals, and an alteration in membrane function is an important component of oxidative stress in cells. Lipid peroxidation of membranes is regulated by the availability of substrate in the form of PUFA, the availability of inducers such as free radicals and excited state molecules to initiate propagation, the antioxidant defense status of environment and the physical status of the membrane lipids (Anandan et al., 1998). In the present study, the level of lipid peroxidation was significantly (P<0.001) increased in the heart tissue of Group III adriamycin-administered rats compared to Group I normal control animals (Figure 1). A parallel reduction in the level of reduced glutathione (Figure 2) and the activities of glutathione-dependent antioxidant enzymes were observed in the heart tissue of Group IV animals.

### Table 1. Antioxidant effect of glutamine on the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the heart tissue of normal and experimental cardiomyopathy induced rats.

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(A): Glutamine, 150mg/kg body weight/day, by intragastric incubation for 15 days.
(B): Adriamycin, 1.5mg/kg body weight/ day, i.p. for 15 days.

Values are expressed as mean ± S.D.; n = 6; ***P < 0.001; Group III vs. Group I; Group IV vs. Group III; Student’s t-test.

### Statistical Analysis

Results are expressed as mean ± SD, and Student’s t-test was used to assess the statistical significance. A P-value <0.05 was considered as statistically significant.

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enzymes and antiperoxidative enzymes (Table 1) in the heart tissue was also observed. This present observation concurs with earlier reports (Daosukho et al., 2007; Gnanapragasam et al., 2004), which showed that myocardial antioxidant defense system was operating at a lower rate despite higher level of oxidative stress in adriamycin-induced cardiomyopathy condition. The adriamycin-induced generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging enzymes to dismute the radicals, resulting in myocyte lesions and reduction of scavengers, as evident from the present study.

Oral administration of glutamine significantly (P<0.001) counteracted the adriamycin-induced lipid peroxidation and also maintained the level of reduced glutathione and the activities of antioxidant enzymes in the heart tissue of Group IV rats at levels comparable to that of control animals. Earlier investigations by Mates et al. (2002) showed that glutamine was used to supply glutamate and cysteine, perhaps for glutathione biosynthesis. Reports by Prem et al. (1999) demonstrated that glutamine preserved total glutathione levels after injury/ischemia. The quenching of reactive oxygen species mediated reactions decreases oxidized protein levels and normalize index enzyme activities. Glutamine via glutamate and glutathione biosynthesis can prevent oxidation of redox of highly sensitive enzymes and thus protects the functions of the myocardium. Studies by Matilla et al. (2000) showed that glutathione concentration was normalized in animals supplemented with glutamine or alanyl glutamine. It has been shown previously that the administration of glutamine-supplemented nutrition protects the liver and improves survival during acetaminophen-induced hepatic injury in rats, an effect probably due to the maintenance of liver glutathione (Hong et al., 1992). The administration of glutamine protects the myocardium from the necrotic damage during adriamycin-induced cardiomyopathy in rats, an effect probably due to the maintenance of the heart glutathione.

**Conclusion**

There is an urgent need to explore various strategies to combat the increasing risk of adriamycin-induced cardiomyopathy. The present result indicates that the protective effect of glutamine against adriamycin-induced cardiotoxicity in rats could be related to its effects on antioxidant defense system. The overall cardioprotective effect of glutamine is probably due to it membrane stabilizing action, or to a counteraction of free radicals by its antioxidant nature, or to its ability to maintain near to the normal status the activities of the free radical scavenging enzymes and the level of reduced glutathione, which protect myocardial membrane against peroxidative damage by decreasing lipid peroxidation and strengthening the myocardial membrane. The present observations highlight that glutamine may be one of the promising drug for improving defense mechanisms in the physiological systems against oxidative stress caused during adriamycin-induced cardiomyopathy.

**REFERENCES**


