

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^\bullet), stimulator of lipid peroxidation and source for destruction and damage to the cell membrane (Zhou et al., 1999). The major abnor-

normalities noticed following adriamycin-induced cardiomyopathy are lipidemia, peroxidation and loss of plasma membrane integrity. A considerable body of clinical and experimental evidence now exists suggesting the involvement of free radical mediated oxidative process in the pathogenesis of adriamycin-induced cardiomyopathy (Bast et al., 2007). Despite this complexity, impressive recent progress has been achieved in advancing our understanding and appreciation of the cellular processes and mechanistic bases underlying cardiac dysfunction associated with adriamycin-induced cardiomyopathy and most importantly, in applying this knowledge to therapeutic interventions.

Glutamine, a multifaceted amino acid used as an energy substrate for most cells, is one of the principal free intracellular amino acids in mammalian heart cells (Rennie et al., 1994). It is important as a constituent of proteins and as a central metabolite for amino acid

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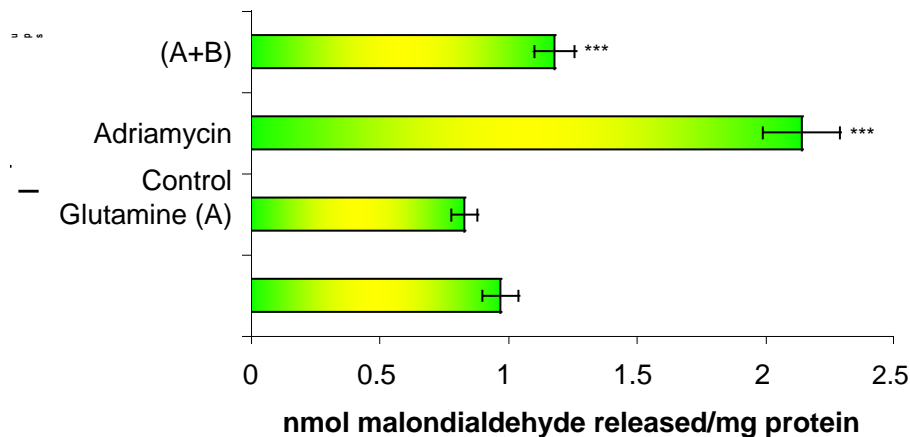


Figure 1. Antioxidant effect of glutamine on the level of lipid peroxidation (LPO) against adriamycin-induced cardiomyopathy in rats (A): Glutamine, 150mg/kg body weight/day, by intragastric intubation for 15 days. (B): Adriamycin, 1.5mg/kg body weight/ day, i.p. for 15 days. Values are expressed as mean \pm S.D.; $n = 6$; *** $P < 0.001$; Group III vs. Group I; Group IV vs. Group III; Student's t -test. Values expressed: LPO, nmol malondialdehyde released/mg protein.

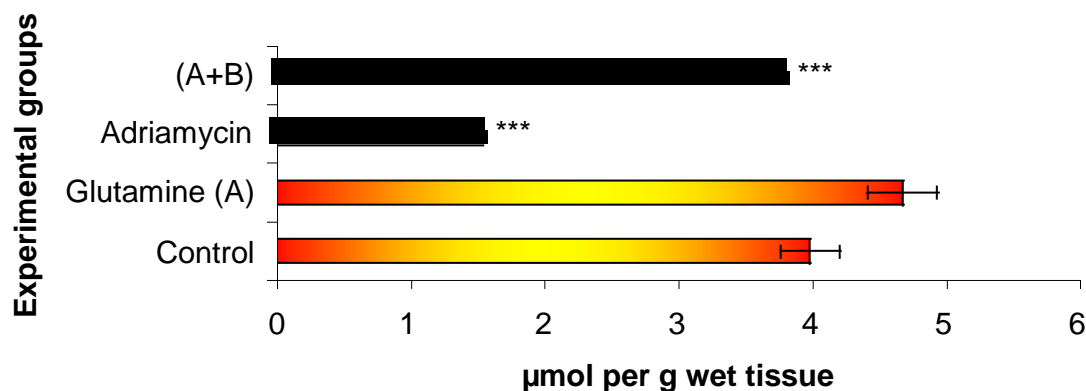


Figure 2. Antioxidant effect of glutamine on the level of reduced glutathione (GSH) in adriamycin-induced cardiomyopathy in rats (A): Glutamine, 150 mg/kg body weight/day, by intragastric intubation for 15 days. (B): Adriamycin, 1.5 mg/kg body weight/ day, i.p. for 15 days. Values are expressed as mean \pm S.D.; $n = 6$; *** $P < 0.001$; Group III vs. Group I; Group IV vs. Group III; Student's t -test. Values expressed: GSH, $\mu\text{mol g}^{-1}$ wet tissue.

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) (Wilndmueller and Spaeth, 1974). Glutamine plays an important role in the nitrogen and carbon-skeleton exchange among different tissues, where this amino acid fulfils many different physiological functions (Kovaccvic and McGivan, 1983). It has immunoregulative 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.

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.

Parameters	Control	Glutamine (A)	Adriamycin (B)	(A+B)
Glutathione-dependent antioxidant enzymes				
GPx	3.25 ± 0.24	3.39 ± 0.27	1.41 ± 0.09 ^{***}	3.07 ± 0.19 ^{***}
GST	1524 ± 127	1598 ± 130	876 ± 65.1 ^{***}	1385 ± 108 ^{***}
Antiperoxidative enzymes				
CAT	15.2 ± 1.04	14.7 ± 0.98	6.25 ± 0.45 ^{***}	14.1 ± 0.91 ^{***}
SOD	6.12 ± 0.44	6.23 ± 0.41	2.57 ± 0.12 ^{***}	5.72 ± 0.38 ^{***}

(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. Values expressed: GPx, nmol GSH oxidized min⁻¹ mg⁻¹ protein; GST, mol 1-chloro-2,4-dinitrobenzene conjugate formed min⁻¹ mg⁻¹ protein; CAT, nmol H₂O₂ decomposed min⁻¹ mg⁻¹ protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation.

MATERIALS AND METHODS

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±2°C, 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 Valentine, 1967), glutathione-S-transferase (GST) (Habig et al., 1974), superoxide dismutase (SOD) (Misra and Fridovich, 1972) and catalase (CAT) (Takahara et al., 1960).

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.

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-administrated 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 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 dismutate 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 its 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

- Anandan R, Devi KP, Devaki T, Govindaraju P (1998). Preventive effects of *Picrorhiza kurroa* on D-galactosamine-induced hepatitis in rats. *J. Clin. Biochem. Nutr.* 25: 87-95.
- Bast A, Haenen GR, Bruynzeel AM, Van der Vijgh WJ (2007). Protection by flavonoids against anthracycline cardiotoxicity: from chemistry to clinical trials. *Cardiovasc. Toxicol.* 7: 154-159.
- Chularojmontri L, Wattanapitayakul SK, Herunsalee A, Charuchongkolwongse S, Niumsukul S, Srichairat S (2005). Antioxidative and cardioprotective effects of *Phyllanthus urinaria* L. on doxorubicin-induced cardiotoxicity. *Biol. Pharm. Bull.* 28: 1165-1171.
- Daosukho C, Chen Y, Noel T, Sompol P, Nithipongvanitch R, Velez JM, Oberley TD, St Clair DK (2007). Phenylbutyrate, a histone deacetylase inhibitor, protects against adriamycin-induced cardiac injury. *Free Radic. Biol. Med.* 42: 1818-1825.
- Ellman GL (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70-77.
- Fox RE, Hopkins IB, Cabacungan ET, Tildon JT (1996). The role of glutamine and other alternate substrates as energy sources in the fetal lung type II cell. *Pediatr. Res.* 40: 135-141.
- Gianni L, Salvatorelli E, Minotti G (2007). Anthracycline cardiotoxicity in breast cancer patients: synergism with trastuzumab and taxanes. *Cardiovasc. Toxicol.* 7: 67-71.
- Gnanapragasam A, Ebenezer KK, Sathish V, Govindaraju P, Devaki T (2004). Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. *Life Sci.* 76: 585-597.
- Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249: 7130-7139.
- Hong RW, Rounds JD, Helton WS, Robinson MK, Wilmore DW (1992). Glutamine preserves liver glutathione after lethal hepatic injury. *Ann. Surg.* 215: 114-119.
- Kim DS, Kim HR, Woo ER, Hong ST, Chae HJ, Chae SW (2005). Inhibitory effects of rosmarinic acid on adriamycin-induced apoptosis in H9c2 cardiac muscle cells by inhibiting reactive oxygen species and the activation of c-Jun N-terminal kinase and extracellular signal-regulated kinase. *Biochem. Pharmacol.* 70: 1066-1078.
- Kovaccvic JD, McGivan (1983). Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol. Rev.* 63: 547-605.
- Mates JM, Gomez CP, Castro GN, Asenjo M, Marquez J (2002). Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/ death. *Int. J. Biochem. Cell. Biol.* 34: 439-558.
- Matilla B, Ortiz J, González P, García-Díez F, Jorquera F, Culebras JM, González-Gallego J, Tuñón MJ (2000). Effects of parenteral nutrition supplemented with glutamine or glutamine dipeptides on liver antioxidant and detoxication systems in rats. *Nutrition.* 16: 125-128.
- Misra HP, Fridovich I (1972). The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem.* 247: 3170-3175.
- Murphy C, Newsholme P (1997). Glutamine as a possible precursor of L- arginine and thus nitric oxide synthesis in murine macrophages. *Biochem. Soc. Trans.* 25: 404S-410S.
- Ohkawa H, Ohishi N., Yagi, K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351- 358.
- Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70: 158-169.
- Prem JT, Eppinger M, Lemmon G, Miller S, Nolan D, Peoples J (1999). The role of glutamine in skeletal muscle ischemia/reperfusion injury in the rat hind limb model. *Am. J. Surg.* 178: 147-150.

- Rajaprabhu D, Rajesh R, Jeyakumar R, Buddhan S, Ganesan B, Anandan R (2007). Protective effect of *Picrorhiza kurroa* on antioxidant defense status in adriamycin-induced cardiomyopathy in rats. *J. Med. Plant Res.* 1: 80-85.
- Rennie MJ, Tadros L, Khogali S, Ahmed A, Taylor P (1994). Glutamine transport and its metabolic effects. *J. Nutr.* 124: 1503S-1508S.
- Roth E, Oehler R, Manhart N, Exner R, Wessner B, Strasser E, Spittler A (2002). Regulative potential of glutamine-relation to glutathione metabolism. *Nutrition.* 18: 217-221.
- Subashini R, Yogeeta S, Gnanapragasam A, Devaki T (2006). Protective effect of *Nardostachys jatamansi* on oxidative injury and cellular abnormalities during doxorubicin-induced cardiac damage in rats. *J. Pharm. Pharmacol.* 58: 257-262.
- Suleiman MS, Fernando C, Dihmis W, Hutter J, Chapman RA (1993). A loss of taurine and other amino acids from ventricles of patients undergoing bypass surgery. *Br. Heart. J.* 69: 241-245.
- Takahara S, Hamilton BH, Nell JV, Ogubra TY, Nishimura ET (1960). Hypocatalasemia: a new genetic carrier state. *J. Clin. Invest.* 39: 610-619.
- Wilndmueller HG, Spaeth AE (1974). Uptake and metabolism of plasma glutamine by the small intestine. *J. Biol. Chem.* 249: 5070-5079.
- Zhou GY, Zhao BL, Hou JW, Ma GE, Xin WJ (1999). Protective effects of sodium tanshinone IIA sulphonate against adriamycin-induced lipid peroxidation in mice hearts in vivo and in vitro. *Pharmacol. Res.* 40: 487-491.