Original Article *J. Clin. Biochem. Nutr.*, **40**, 49–55, January 2007

Biochemical Studies on the Cardioprotective Effect of Glutamine on Tissue Antioxidant Defense System in Isoprenaline-Induced Myocardial Infarction in Rats

Subramaniam H.S. Kumar[1,2] and Rangasamy Anandan[2,*]

[1]*Department of Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500 072, INDIA*
[2]*Biochemistry and Nutrition Division,Central Institute of Fisheries Technology, Matsyapuri (PO),Cochin-682 029, INDIA*

Received: 24 April, 2006; Accepted 7 August, 2006

Summary **Oxidative stress is one of the mechanisms with a central role involved in the pathogenesis of myocardial infarction. The protective effect of glutamine on myocardial antioxidant defense system was investigated during isoprenaline-induced myocardial infarction, an animal model of myocardial infarction of human beings. Levels of diagnostic marker enzymes in plasma, reduced glutathione (GSH) and lipid peroxides and the activities of glutathione peroxidase, glutathione-S-transferase, catalase and superoxide dismutase in heart tissue were determined. Injection of isoprenaline caused significant increases in the levels of diagnostic marker enzymes in plasma and lipid peroxidation in heart tissue. A parallel decline in the levels of ATP (Adenosine triphosphate) and GSH and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes in heart tissue was also observed. Prior oral administration of glutamine significantly prevented isoprenaline-induced adverse effects and maintained myocardial antioxidant status at near normal status. The cardioprotective effect of glutamine is probably related to a strengthening of the myocardial membrane by its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant property, or to its ability to maintain near to normal status the activities of free radical scavenging enzymes and the level of GSH, which protect myocardial membrane against oxidative damage by decreasing lipid peroxidation.**

Key Words: **glutamine, isoprenaline, myocardial infarction, diagnostic marker enzymes, antioxidant status**

Introduction

Myocardial infarction and the resultant abnormalities in cardiac function represent the leading cause of morbidity and mortality in developed countries [*1*]. However, with changing life style in developing countries, like India, particularly in urban areas, myocardial infarction is making an increasingly important contribution to mortality statistics of such countries [*2*]. It is well recognized that myocardial infarction is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart [*3*]. 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 myocardial infarction and most

*To whom correspondence should be addressed.
Tel: +91-484-2666845 Fax: +91-484-2668212
E-mail: kranandan@email.com

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 [*4*]. It is important as a constituent of proteins and as a central metabolite for amino acid 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) [*5*]. Glutamine plays an important role in the nitrogen and carbon-skeleton exchange among different tissues, where this amino acid fulfils many different physiological functions [*6*]. It has immuno-regulative and cell-regulative capabilities, as recent investigations have shown [*7*]. It also regulates endothelial nitric oxide metabolism in the heart tissue [*8*]. One of the most important characteristics of glutamine is that it plays a critical role in glutathione biosynthesis by providing glutamate to the glutathione system, which is one of the main sources of the antioxidant defense system in the cell [*9*]. It is also involved in cell membrane stabilization, antioxidation, detoxification, and energy production [*4*, *10*, *11*]. Significant reduction in the intracellular concentration of glutamine has been reported to occur in the heart tissue during myocardial infarction condition [*12*]. Though glutamine is assumed to participate in various important biological and physiological functions in heart, the protective effect of glutamine on myocardial antioxidant defense system in experimentally induced myocardial infarction condition has not been explored in detail.

Myocardial infarction induced by isoprenaline [isoproterenol; L-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride], a β-adrenergic agonist, shows many metabolic and morphologic aberrations in the myocardium of experimental animals similar to those observed in myocardial infarction in man [*13*]. Intraperitoneal administration of isoprenaline produces necrotic lesions and increases lipid peroxidation in the myocardium, which plays a significant part in the pathogenesis of myocardial dysfunction [*14*]. Alterations in the activities of antiperoxidative enzymes [superoxide dismutase (SOD) and catalase (CAT)] and glutathione dependent antioxidant enzymes [glutathione peroxidase (GPX) and glutathione-S-transferase (GST)] have been reported in experimentally induced myocardial infarction in rats [*15*].

In the present study, we have investigated the cardioprotective effects of glutamine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats by virtue of its membrane stabilizing and antioxidant properties.

Materials and Methods

Chemicals

Epinephrine, glutamine, isoprenaline and tetraethoxy propane were obtained from M/s. Sigma Chemical Company, St. Louis. MO. All the other chemicals used were of analytical grade.

Animals

Male Wistar strain albino rats, weighing 120–150 g were selected for the study. The animals were housed individually in polyurethane 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 and approved by the Institutional Animal Ethics Committee (IAEC).

Induction of myocardial infarction

The myocardial infarction was induced in experimental rats by injecting isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body weight day^{-1}], i.p. for 2 days [*16*].

Experimental protocol

The experimental animals were divided into four groups, comprising six rats each. Rats in Group I (normal control) received standard diet for a period of 20 days. Group II animals were orally administered with glutamine [100 mg (dissolved in distilled water)/kg body weight/day] by intragastric intubation for a period of 20 days. In Group III, rats were injected with isoprenaline [11 mg (dissolved in physiological saline) 100 g^{-1} body wt day^{-1}], i.p. for 2 days for the induction of myocardial infarction. In Group IV, the animals were pretreated with glutamine [100 mg/kg body weight/day, p.o for 20 days before the induction of myocardial infarction as described for Group III. Control animals (Group I and Group II) were injected with physiological saline alone for 2 days.

At the end of the experimental period, i.e., 24 h after last injection of isoprenaline, the experimental animals were sacrificed blood was collected using heparin as anticoagulant and the plasma separated was used for the determination of diagnostic marker enzymes. The heart tissue was excised immediately and washed with chilled isotonic saline. The heart tissue homogenates prepared in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH) and antioxidant enzymes.

Biochemical assays

The activities of alanine aminotransferase (ALT) [EC

2.6.1.2] and aspartate aminotransferase (AST) [EC 2.6.1.1] in plasma were estimated by the method of Mohur and Cook [*17*]. The lactate dehydrogenase (LDH) [EC 1.1.1.27] activity in plasma was determined spectrophotometrically according to the method of King [*18*]. The creatine phosphokinase (CPK) [EC 2.7.3.2] activity in plasma was assayed by the method of Okinaka *et al*. [*19*]. The level of ATP in the heart tissue was determined by the method of Ryder [*20*] using Shimadzu LC 10 ATvp HPLC. Myocardial lipid peroxides level was determined as TBA-reactive substances by the method of Ohkawa *et al.* [*21*] and the GSH content was estimated by the method of Ellman [*22*]. The method described by Paglia and Valentine [*23*] was followed for the assay of glutathione peroxidase [EC 1.11.1.9] activity and glutathione-S-transferase (GST) [EC 2.5.1.18] activity was determined by the method of Habig *et al*. [*24*]. Catalase (CAT) [EC 1.11.1.6] activity was determined by the method of Takahara *et al*. [*25*] and the method described by Misra and Fridovich [*26*] was adopted for the determination of superoxide dismutase (SOD) [EC 1.15.1.1] activity. The protein content was determined by the method of Lowry *et al*. [*27*].

Statistical analysis

Results are expressed as mean ± SD. Multiple comparisons of the significant ANOVA were performed by Tukey's multiple comparison test. A p-value <0.05 was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

Results

Table 1 depicts the levels of diagnostic marker enzymes (AST, ALT, LDH and CPK) in the plasma of normal and experimental groups of rats. There was a significant (p<0.001) increase noticed in the levels of these marker enzymes in the plasma of Group III isoprenaline-induced myocardial infarcted rats as compared to that of Group I control rats. The oral pre-treatment with glutamine significantly (p<0.001) prevented the isoprenaline-induced release of these enzymes from the myocardium into the systemic circulation and maintained the rats at near normal status, indicating the cytoprotective action of glutamine. Figure 1 shows the level of myocardial ATP content in normal and experimental groups of rats. Significant (p<0.001) reduction in the level of ATP content in the heart tissue of Group III myocardial infarction induced rats as compared to that of Group I control animals. The prior administration of glutamine maintained the level of ATP at near normalcy in Group IV rats as compared to that of Group III myocardial infarction induced rats, reflecting its ability to maintain the function of the heart mitochondria at near normal status.

Table 2 depicts the levels of lipid peroxides and reduced glutathione and the activities of glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes in the heart tissue of normal and experimental groups of rats. There was a significant (p<0.001) increase in the level of lipid peroxidation along with a concomitant decline in the level of GSH noted in the heart tissue of Group III isoprenaline-administered rats as compared to controls. Also a significant (p<0.001) reduction in the activities of glutathione dependent antioxidant enzymes (GPx and GST)

Table 1. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) in plasma of normal and experimental groups of rats.

Parameters	Control	Glutamine (A)	Isoprenaline (B)	(A + B)
ALT	92.4 ± 8.71	90.8 ± 8.25	349 ± 27.6[a,b]	118 ± 9.54[c,d,e]
AST	89.3 ± 6.42	82.7 ± 6.86	376 ± 23.9[a,b]	124 ± 8.35[c,f,g]
LDH	176 ± 12.2	182 ± 11.7	345 ± 21[a,b]	198 ± 14.2[c,d,e]
CPK	125 ± 8.54	118 ± 9.23	327 ± 17.1[a,b]	143 ± 9.7[c,d,e]

(A): Glutamine, 100 mg/kg body wt/day, p.o. for 20 days.
(B): Isoprenaline, 11 mg/100 g body wt/day, i.p. for 2 days.

Results are mean ± SD of six animals. Values expressed: ALT, AST, and LDH, μmol pyruvate liberated $h^{-1}l^{-1}$; CPK, μmol creatine liberated $h^{-1}l^{-1}$. [a]p<0.001 significantly different compared with control animals; [b]p<0.001 significantly different compared with glutamine-administered normal rats; [c]p<0.001 significantly different compared with isoprenaline-induced myocardial infarcted rats; [d]p<0.05 significantly different compared with control animals; [e]p<0.05 significantly different compared with glutamine-administered normal rats; [f]p<0.01 significantly different compared with control rats; [g]p<0.01 significantly different compared with glutamine-administered normal rats.

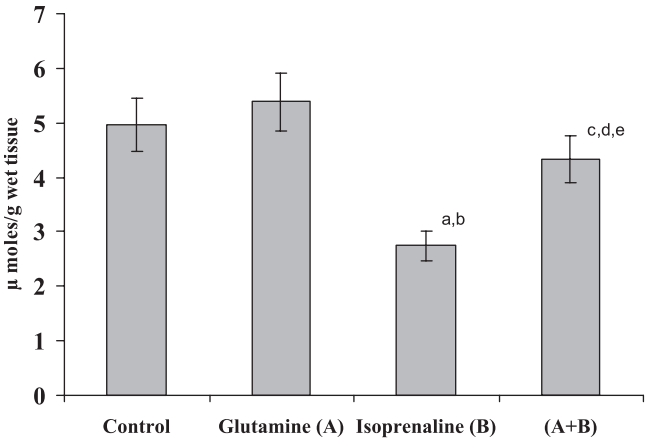

Fig. 1. Level of ATP in the heart tissue of control and experimental groups of rats.
(A): Glutamine, 100 mg/kg body wt/day, p.o. for 20 days.
(B): Isoprenaline, 11 mg/100 g body wt/day, i.p. for 2 days.

Results are mean ± SD for 6 animals. [a]$p<0.001$ significantly different compared with Group 1 control animals; [b]$p<0.001$ significantly different compared with Group 2 glutamine-administered normal rats; [c]$p<0.001$ significantly different compared with Group 3 isoprenaline-induced myocardial infarcted rats; [d]$p<0.01$ significantly different compared with Group 2 animals; [e]$p<0.05$ significantly different compared with Group 1 animals.

and antiperoxidative enzymes (SOD and CAT) was observed. The prior administration of glutamine significantly reduced the isoprenaline induced adverse effects and maintained the level of evaluated parameters at near normalcy. In Group II rats the oral administration of glutamine resulted in a significant ($p<0.01$) elevation in the level of reduced glutathione.

Discussion

The focus of the current study was to evaluate the effects of glutamine for its antioxidant and membrane-stabilizing properties during experimentally induced myocardial injury. In the present study, there was a significant ($p<0.001$) increase noticed in the levels of diagnostic marker enzymes (ALT, AST, LDH and CPK) in the plasma of Group III myocardial infarction induced rats as compared to that of Group I control rats, suggesting the occurrence of considerable necrotic damage to the myocardial membrane. This finding concurs with a previous reported study [*28*], which indicates that of all the macromolecules leaking from the damaged cardiac tissue, these enzymes because of their tissue specificity and catalytic activity are the best markers of myocardial infarction. The release of these marker enzymes reflects a non-specific aberration in the structural and functional integrity of myocardial membrane as a response to the β-adrenergic stimulation.

In the present study, the prior administration of glutamine was found to significantly ($p<0.001$) reduce the release of the cytosolic enzymes into the systemic circulation, thereby demonstrating its cytoprotective action on the cell membranes. The viability of the myocardial cell depends for the most part on the integrity of several vital subcellular systems and the major source of energy for contraction comes from the oxidative metabolism of mitochondria in the myocardial cell. Glutamine is utilized as a major energy source and drives mitochondrial ATP formation [*29*]. The first step for glutamine catabolism in the mitochondria is its transport through the inner mitochondrial membrane by a protein-catalyzed process [*30*]. Sudden depletion of glutamine has been reported to result in a sharp decline in mitochondrial respiration [*31*]. Our observations also confirm the same pattern and shows significant ($p<0.001$) decline in the level of myocardial ATP content in Group III rats as compared to that of Group I control animals. Ischemic cells are known to utilize glutamate for energy production and a fall in tissue glutamate does occur in the hearts of patients undergoing coronary artery bypass surgery [*12*]. In the present study, the prior administration of glutamine maintained the level of ATP at near normalcy in Group IV rats as compared to that of Group III myocardial infarction induced rats, reflecting its ability to maintain the function of the heart mitochondria at near normal status. It is possible therefore that in the present study, the conversion of glutamine to glutamate might have provided the much-needed substrate for the Krebs cycle for energy production to counteract isoprenaline-induced membrane disintegration in the myocardium. Reports by Dumaswala *et al.* [*32*] indicated that glutamine could exert protective effects on membrane structure by preserving transmembrane ion gradient and lipid asymmetry.

Oxidative stress is the result of excessive production of oxygen species and/or depletion of intracellular antioxidant defenses, leading to imbalance in the redox status of the cell. A growing body of evidence is emerging which suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of myocardial infarction [*14*]. The effects of oxidative stress can be evidenced by cellular accumulation of lipid peroxides. In the present study, a significant rise in the level of lipid peroxides was noted in the heart tissue of Group III isoprenaline-administered rats as compared to that of Group I normal rats. Our findings are in accordance with earlier reported investigations [*15*], which indicated that isoprenaline upon oxidation produces $O_2^{-\cdot}$ and H_2O_2. Peroxidation of endogenous lipids has been shown to be a major factor in the cardiotoxic action of isoprenaline [*33*]. Reports by Nirmala and Puvanakrishnan [*34*] indicated that lack of antioxidant defense might have resulted in increased lipid peroxidation and subsequent deleterious effects on the cardiac membrane in isoprenaline-induced myocardial

Table 2. Levels of lipid peroxides (LPO) and reduced glutathione (GSH) and the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the heart tissue of normal and experimental groups of rats.

Parameters	Control	Glutamine (A)	Isoprenaline (B)	(A + B)
LPO	1.08 ± 0.05	0.87 ± 0.06	2.32 ± 0.18[a,b]	1.14 ± 0.08[c,d,e]
GSH	4.76 ± 0.24	6.49 ± 0.37[g]	2.32 ± 0.11[a,b]	5.92 ± 0.45[c,e,f]
GPx	2.43 ± 0.18	2.76 ± 0.24	1.15 ± 0.07[a,b]	2.30 ± 0.15[c,e]
GST	1256 ± 94	1345 ± 105	812 ± 64[a,b]	1118 ± 92[c,e]
CAT	8.45 ± 0.52	8.93 ± 0.61	3.74 ± 0.21[a,b]	7.67 ± 0.58[c,d,e]
SOD	4.15 ± 0.27	4.33 ± 0.25	1.78 ± 0.12[a,b]	3.83 ± 0.17[c,e]

(A): Glutamine, 100 mg/kg body wt/day, p.o. for 20 days.
(B): Isoprenaline, 11 mg/100 g body wt/day, i.p. for 2 days.

Results are mean ± SD for 6 animals. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, μmol g^{-1} wet tissue; GPx, nmol GSH oxidized $min^{-1}mg^{-1}$ protein; GST, μmol 1-chloro-2,4-dinitrobenzene conjugate formed $min^{-1}mg^{-1}$ protein; CAT, nmol H_2O_2 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 [a]$p<0.001$ significantly different compared with control animals; [b]$p<0.001$ significantly different compared with glutamine-administered normal rats; [c]$p<0.001$ significantly different compared with isoprenaline-induced myocardial infarcted rats; [d]$p<0.05$ significantly different compared with control animals; [e]$p<0.05$ significantly different compared with glutamine-administered normal rats; [f]$p<0.01$ significantly different compared with control rats; [g]$p<0.001$ significantly different compared with glutamine-administered normal rats.

infarction condition.

In the present study, the rats pre-administered with glutamine showed significant ($p<0.001$) reduction in the level of lipid peroxidation in the heart tissue of Group IV rats as compared with that of Group III myocardial infarction induced rats, indicating the antioxidant property of glutamine against isoprenaline-induced lipid peroxidation. The possible antioxidant effects of glutamine occur through the formation of nitric oxide. Earlier reports [*35*] indicated that nitric oxide derived from donor compounds showed marked protection against the cytotoxic effects of both hydrogen peroxide and alkyl hydroperoxide as well as hypoxanthine/xanthine oxidase-mediated cytotoxicity. NO released from glutamine either scavenges or prevents the formation of reactive oxygen species derived from hydrogen peroxide and superoxide [*36*]. It can rapidly react with superoxide anion ($O_2^{-\cdot}$), which is subsequently protonated to form $OH^{\cdot}$. Previous experimental evidence [*37*], which supports the role of NO formed from glutamine as an antioxidant, indicates that the affinity of NO for $O_2^{-\cdot}$ is greater than that of SOD for $O_2^{-\cdot}$. In fact NO from glutamine even competes with SOD for $O_2^{-\cdot}$ supporting its role as an antioxidant. Direct action between NO and lipid peroxyl radical forms a lipid nitroso adduct and this radical-radical interaction results in the termination of lipid peroxidation chain reaction and stops further initiation [*38*]. Earlier Gonzales *et al.* [*39*] reported the antioxidant property of glutamine against cobalt-induced lipid peroxidation in rat liver.

Glutathione exerts protective antioxidant functions by reaction with superoxide radicals and peroxy radicals, followed by the formation of oxidized glutathione and other disulphides. In the present study, a significant reduction in the level of reduced glutathione was observed in the heart tissue of Group III isoprenaline-administered rats as compared to that of Group I control rats. This was paralleled by significant decline in the activities of glutathione-dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) in the heart tissue of Group III myocardial infarction induced rats. This is consistent earlier reported studies [*15*, *40*], which indicated that reduction in the activities of enzymatic and non-enzymatic scavengers makes myocardial membrane more susceptible isoprenaline-mediated necrotic damage. Decreases in the activities of antioxidant enzymes leads to the formation of $O_2^{-\cdot}$ and H_2O_2, which in turn form hydroxyl radical ($OH^{\cdot}$) and bring about a number of reactions harmful to the cellular and subcellular membranes in the myocardium.

In the present study, the prior oral administration of glutamine significantly prevented the isoprenaline-induced depletion of reduced glutathione and maintained the activities of antioxidant enzymes in the heart tissue of Group IV rats at near normalcy. A significant rise ($p<0.001$) in the level of GSH was noticed in the heart tissue in Group II glutamine administered rats as compared to that Group I control rats,

indicating that tissue antioxidant status was operating at a higher rate in glutamine-treated rats for the counteraction of lipid peroxides. This finding is in line with an earlier report [*41*], which has shown that glutamine is an efficient precursor of glutathione. There is an experimental basis for the concept that glutamine may augment the glutathione system in combating oxidative stress of tissue injury. Models of gut and liver injury have suggested that supplying an adequate amount of glutamine can augment the glutathione system and its ability to combat ischemia reperfusion injury [*42*]. Previous studies [*43*] have also indicated that pre-injury augmentation of total glutathione may reduce cellular injury. It has been shown previously that the administration of glutamine-supplemented nutrition protects the liver and improves the survival during acetaminophen-induced hepatic injury in rats, an effect probably due to the maintenance of liver glutathione [*9*]. It is also possible that the glutamine itself may have provided the protective role. Reports by Karinch *et al*. [*44*] have shown that during inflammatory process, concentrations of glutamine and glutathione are augmented within the endothelial cell. Our results lead to the conclusion that a relation may exist between those protective effects of glutamine and glutathione biosynthesis because glutathione is a tripeptide consisting of glutamate, cysteine and glycine.

In conclusion, the results of the present study indicate that the prior administration of glutamine attenuates isoprenaline-induced myocardial infarction in rats. The cardioprotective effect of glutamine is probably related to a strengthening of the myocardial membrane by its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant property, or to its ability to maintain near to normal status the activities of free radical scavenging enzymes and the level of GSH, which protect myocardial membrane from oxidative damage by decreasing lipid peroxidation.

References

[1] Chada, S.L.: Urban-rural differences in prevalence of coronary heart disease and its risk factors. *Curr. Sci.*, **74**, 1069–1073, 1998.

[2] Levy, R.I. and Feinleib, M.: Heart disease, *in A Text Book of Cardiovascular Medicine, vol 2*, eds. By Brawnwald, E., Philadelphia: W.B. Saunders Co., pp. 1205–1234, 1984.

[3] Petrich, E.R., Schanne, O.F., and Zumino, A.P.: Electrophysiological responses to ischemia and reperfusion, *in Myocardial Ischemia: Mechanism, Reperfusion, Protection*, eds. By Karmazyn, M., Basel: Birkhauser Verlag, pp. 115–133, 1996.

[4] Rennie, M.J., Tadros, L., Khogali, S., Ahmed, A., and Taylor, P.: Glutamine transport and its metabolic effects. *J. Nutr.*, **124**, 1503S–1508S, 1994.

[5] Wilndmueller, H.G. and Spaeth, A.E.: Uptake and metabolism of plasma glutamine by the small intestine. *J. Biol. Chem.*, **249**, 5070–5079, 1974.

[6] Kovaccvic, J. D. and McGivan, J.D.: Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol. Rev.*, **63**, 547–605, 1983.

[7] Roth, E., Oehler, R., Manhart, N., Exner, R., Wessner, B., Strasser, E., and Spittler, A.: Regulative potential of glutamine-relation to glutathione metabolism. *Nutrition*, **18**, 217–221, 2002.

[8] Murphy, C. and Newsholme, P.: Glutamine as a possible precursor of L-arginine and thus nitric oxide synthesis in murine macrophages. *Biochem. Soc. Trans.*, **25**, 404S–410S, 1997.

[9] Hong, R.W., Rounds, J.D., and Helton, W.S.: Glutamine preserves liver glutathione after lethal hepatic injury. *Ann. Surg.*, **215**, 114–119, 1992.

[10] Matilla. B., Ortiz. J., Gonzalez, P., Diez, F.G., Jorquera, F., Culebras, J.M., Gallego, J.G., and Tunon, M.I.: Effects of parenteral nutrition supplemented with glutamine or glutamine dipeptides on liver antioxidant and detoxification system in rats. *Nutrition*, **16**, 125–128, 2000.

[11] Fox, R.E., Hopkins, I.B., Cabacungan, E.T., and Tildon, J.T.: The role of glutamine and other alternate substrates as energy sources in the fetal lung type II cell. *Pediatr. Res.*, **40**, 135–141, 1996.

[12] Suleiman, M.S., Fernando, C., Dihmis, W., Hutter, J., and Chapman, R.A.: A loss of taurine and other amino acids from ventricles of patients undergoing bypass surgery. *Br. Heart. J.*, **69**, 241–245, 1993.

[13] Geng, B., Chang, L., Pan, C., Qi, Y., Zhao, J., Pang, Y., Du, J., and Tang, C.: Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. *Biochem. Biophys. Res. Commun.*, **318**, 756–763, 2004.

[14] Rathore, N., John, S., Kale, M., and Bhatnagar, D.: Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol. Res.*, **38**, 297–303, 1998.

[15] Farvin, K.H.S., Anandan, R., Kumar, S.H.S., Shiny, K.S., Sankar, T.V., and Thankappan, T.K.: Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. *Pharmacol. Res.*, **50**, 231–236, 2004.

[16] Anandan, R., Asha, K.K., Ammu, K., Suseela, M., and Nair, P.G.V.: Effects of peroxidised PUFA on tissue defense system in experimentally induced myocardial infarction in rats, *in Seafood Safety*, eds. By Surendran, P.K., Mathew, P.T., Thampuran, N., Nambiar, N., Joseph, J., Boopendranath, M.R., Laksmanan, P.T., and Nair, P.G.V. Cochin: Society of Fisheries Technologists (India), pp. 330–335, 2003.

[17] Mohur, A. and Cook, I.Y.: Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic-pyruvic transaminase in routine laboratories. *J. Clin. Pathol.*, **10**, 394–399, 1957.

[18] King, J.: The dehydrogenase of oxidoreductase -lactate dehydrogenase, *in Practical Clinical Enzymology*, eds. By King, J.C. and Van, D., London: Nostrand Co., pp. 83–93, 1965.

[19] Okinaka, S., Kumogai, H., Ebashi, S., Sugaita, H., Momoi, H., Toyokura, Y., and Fujie, Y.: Serum creatine phosphokinase activity in progressive muscular dystrophy and

neuromuscular disease. *Arch. Neurol.*, **4**, 520–525, 1961.

[20] Ryder, J.M.: Determination of adenosine triphosphate and its breakdown products in fish muscle by high performance liquid chromatography. *J. Agric. Food. Chem.*, **33**, 678–680, 1985.

[21] Ohkawa, H., Ohishi, N., and Yagi, K.: Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.*, **95**, 351–358, 1979.

[22] Ellman, G.L.: Tissue sulfhydril groups. *Arch. Biochem. Biophys.*, **82**, 70–77, 1959.

[23] Paglia, D.E. and Valentine, W.N.: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, **70**, 158–169, 1967.

[24] Habig, W.H., Pabst, M.J., and Jakoby, W.B.: Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, **249**, 7130–7139, 1974.

[25] Takahara, S., Hamilton, B.H., Nell, J.V., Ogubra, T.Y., and Nishimura, E.T.: Hypocatalasemia: a new genetic carrier state. *J. Clin. Invest.*, **39**, 610–619, 1960.

[26] Misra, H.P. and Fridovich, I.: The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. *J. Biol. Chem.*, **247**, 3170–3175, 1972.

[27] Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275, 1951.

[28] Hearse, D.J.: Cellular damage during myocardial ischemia: metabolic changes leading to enzyme leakage, *in Enzymes In Cardiology*, eds. By Hearse, D.J., Leiris, J.D., and Loisance, D. New York: John Wiley and Sons Ltd., pp. 1–21, 1979.

[29] Piva, T.J. and McEvoy, B.E.: Oxidation of glutamine in HeLa cells: role and control of truncated TCA cycles in tumour mitochondria. *J. Cell. Biochem.*, **68**, 213–225, 1998.

[30] Mates, J.M., Gomez, C.P., Castro, G.N., Asenjo, M., and Marquez, J.: Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. *Int. J. Biochem. Cell. Biol.*, **34**, 439–558, 2002.

[31] Darmaun, D.: Role of glutamine depletion in severe illness. *Diabetes Nutr. Metab.*, **13**, 25–30, 2000.

[32] Dumaswala, U.J., Rugg, N., and Greenwalt, T.J.: Studies in red blood cell preservation. 9. The role of glutamine in red blood cell preservation. *Vox. Sang.*, **67**, 255–258, 1994.

[33] Kumar, S.H.S., Anandan, R., Devaki, T., and Kumar, M.S.: Cardioprotective effects of *Picrorhiza kurroa* against isoproterenol-induced myocardial stress in rats. *Fitoterapia.*, **72**, 402–405, 2001.

[34] Nirmala, C. and Puvanakrishnan, R.: Protective role of curcumin against isoproterenol induced myocardial infarction in rats. *Mol. Cell. Biochem.*, **159**, 85–93, 1996.

[35] Wink, D.A., Osawa, Y., Darbyshire, J.F., Jones, C.R., Eshenaur, S.C., and Nims, R.W.: Inhibition of cytochrome p450 by nitric oxide and a nitric oxide releasing agent. *Arch. Biochem. Biophys.*, **300**, 115–123, 1993.

[36] Maulik, N., Engelman, D.T., Watanabe, M., Engelman, R.M., Rousou, J.A., and Flack, J.E.: Nitric oxide/carbon monoxide. A molecular switch for myocardial preservation during ischemia. *Circulation*, **94**, 398–406, 1996.

[37] Wink, D.A., Hanbauer, I., Krishna, M.C., DeGraff, W., Gamson, J., and Mitchell, J.B.: Nitric oxide protects against cellular damage and cytotoxicity from reactive oxgen species. *Proc. Natl. Acad. Sci.*, **90**, 9813–9817, 1993.

[38] Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M., and Freeman, B.A.: Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen containing oxidized lipid derivatives. *J. Biol. Chem.*, **269**, 26066–26075, 1994.

[39] Gonzales, S., Polizio, A.H., Erario, M.A., and Tomaro, M.L.: Glutamine is highly effective in preventing *in vivo* cobalt-induced oxidative stress in rat liver. *World. J. Gastroenterol.*, **11**, 3533–3538, 2005.

[40] Shiny, K.S., Kumar, S.H.S., Farvin, K.H.S., Anandan, R., and Devadasan, K.: Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. *J. Pharm. Pharmacol.*, **57**, 1–5, 2005.

[41] Cao, Y., Feng, Z., Hoos, A., and Klimberg, V.S.: Glutamine enhances gut glutathione production. *J.P.E.N.*, **22**, 224–227, 1998.

[42] Prem, J.T., Eppinger, M., Lemmon, G., Miller, S., Nolan, D., Peoples, J., and Ohio, D.: The role of glutamine in skeletal muscle Ischemia/Reperfusion injury in the rat hind limb model. *American. J. Sur.*, **178**, 147–150, 1999.

[43] Harward, R.S., Coe. D., and Souba, W.: Glutamine preserves gut glutathione levels during intestinal ischemia/reperfusion. *J. Surg. Res.*, **56**, 351–355, 1994.

[44] Karinch, A.M., Pan, M., Lin, CM., Strange, R., and Souba, W.W.: Glutamine metabolism in sepsis and infection. *J. Nutr.*, **131**, 2535S–2538S, 2001.