Protective effect of betaine on changes in the levels of lysosomal enzyme activities in heart tissue in isoprenaline-induced myocardial infarction in Wistar rats

Balaraman Ganesan · Rangasamy Anandan

Received: 7 January 2009 / Accepted: 6 March 2009 / Published online: 18 March 2009
© Cell Stress Society International 2009

Abstract Myocardial infarction is one of the most common manifestations of cardiovascular disease. In the present study, we investigated the protective effect of betaine, a potent lipotropic molecule, on changes in the levels of lysosomal enzymes and lipid peroxidation in isoprenaline-induced myocardial infarction in Wistar rats, an animal model of myocardial infarction in man. Male albino Wistar rats were pretreated with betaine (250 mg/kg body weight) daily for a period of 30 days. After the treatment period, isoprenaline (11 mg/100 g body weight) was intraperitoneally administered to rats at intervals of 24 h for 2 days. The activities of lysosomal enzymes (β -glucuronidase, β -galactosidase, β -glucosidase, and acid phosphatase) were significantly ($p < 0.05$) increased in plasma with a concomitant decline in the activities of these enzymes in heart tissue of isoprenaline-administered rats. Also, the level of lipid peroxidation was higher in heart lysosomes of isoprenaline-injected rats. Pretreatment with betaine daily for a period of 30 days to isoprenaline-induced rats prevented the changes in the activities of these lysosomal enzymes. Oral treatment with betaine (250 mg/kg body weight) to normal control rats did not show any significant effect in all the biochemical parameters studied. Thus, the results of our study show that betaine protects the

lysosomal membrane against isoprenaline-induced myocardial infarction. The observed effects might be due to the free radical-scavenging and membrane-stabilizing properties of betaine.

Keywords Betaine · Isoprenaline · Myocardial infarction · β -galactosidase · Lipid peroxides

Introduction

Myocardial infarction is one of the most common manifestations of cardiovascular disease. Morbidity and mortality due to myocardial infarction are now reaching epidemic proportions throughout the world, accounting for 16.7 million deaths per year worldwide (Yusuf et al. 2001). It is estimated that by the year 2020, up to three quarters of deaths in developing countries will result from noncommunicable diseases and that myocardial infarction will top the list of killers (Gupta and Gupta 1998). With changing lifestyles in developing countries like India, particularly in urban areas, myocardial infarction is making an increasingly important contribution to mortality statistics (Farvin et al. 2004). In India, the number of patients hospitalized for myocardial infarction has increased over the past 35 years, more strikingly among male patients. It is predicted that by the year 2020, India will have the highest incidence of myocardial infarction in the world (Krishnaswami 1998). There are an estimated 45 million patients with coronary heart disease in India. This increased prevalence of myocardial infarction is largely due to the adoption of “western” lifestyle and its accompanying risk factors such as smoking, high-fat diet, obesity, and lack of exercise.

Natural products have been the starting point for the discovery of many important modern drugs. This fact has

B. Ganesan
Vinayaka Missions University,
Ariyanoor,
Salem 636308 Tamil Nadu, India

B. Ganesan (✉) · R. Anandan
Biochemistry and Nutrition Division,
Central Institute of Fisheries Technology,
Matsyapuri (PO),
Cochin 682029, India
e-mail: ganesancift@rediffmail.com

led to chemical and pharmacological investigations and general biological screening programs for natural products all over the world (Nagle and Zhou 2006). Betaine is a naturally occurring product present in relatively large quantities in sugar beet and aquatic invertebrates (Saunderson and Mackinlay 1990). Betaine (trimethylglycine) (Fig. 1) is a methylated amino acid having a dual role as an osmolyte and as a methyl group donor in vertebrates. Betaine has been found to protect the liver from the damaging effects of CCl_4 (Junnala et al. 2000). Betaine is nonperturbing to cellular metabolism, highly compatible with enzyme function, and stabilizes cellular metabolic function under different kinds of stress in various organisms and animal tissues (Hanson et al. 1994). It has been shown that betaine regulates erythrocyte (red blood cell) membrane ATPases via conformational changes, which results in cell volume control (Moeckel et al. 2002). Earlier, we reported the protective effect of betaine on antioxidant status (Ganesan et al. 2008b), mitochondrial function (Ganesan et al. 2007a), protein and glycoprotein metabolism (Ganesan et al. 2007b), and lipid metabolism (Ganesan et al. 2008a) in isoprenaline-induced myocardial infarction in Wistar rats. In the present study, an attempt was made to assess the protective effects of betaine on isoprenaline-induced myocardial infarction with respect to changes in the levels of lysosomal enzymes and lipid peroxidation.

Isoprenaline[1-(3',4'-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride], (Fig. 2) a synthetic catecholamine and β -adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct-like necrosis of the heart muscle. Isoprenaline-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs and cardiac function (Ithayarasi and Devi 1997). Isoprenaline is also well-known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane (Kakreja and Hess 1992). Isoprenaline-induced myocardial infarction results in increased lysosomal hydrolases activity that may be responsible for tissue damage and infarcted heart (Ravichandran et al. 1991). Intracellular release of lysosomal enzymes

Fig. 1 Chemical structure of betaine

Fig. 2 Structure of isoprenaline hydrochloride

following myocardial ischemia may directly, or through activation of the complement pathway, result in cell injury and death. Isoprenaline-induced myocardial necrosis involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes (Todd et al. 1980).

Materials and methods

Chemicals

Isoprenaline (isoproterenol) and betaine were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats, weighing 150–180 g, were selected for the study. The animals were housed individually in polypropylene cages (with stainless steel grill top) under hygienic and standard environmental conditions ($28 \pm 2^\circ\text{C}$, humidity 60–70%, 12 h light/dark cycle). The animals were allowed a standard diet (Sai Feeds, Bangalore, India) and water ad libitum. The pellet diet consisted of 22.0% crude protein, 4.2% crude oil, 3.0% crude fiber, 7.5% ash, 1.4% sand silica, 0.8% calcium, 0.6% phosphorus, 2.5% glucose, 1.8% vitamins, and 56.2% nitrogen-free extract (carbohydrates). The diet provided metabolizable energy of 3,600 kcal. The experiment was carried out according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Institutional Animal Ethical Committee of the Central Institute of Fisheries Technology.

Induction of myocardial infarction

Myocardial infarction was induced in experimental rats by intraperitoneal (i.p.) injection of isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight/day] for 2 days (Anandan et al. 2003).

Experimental protocol

Five days after acclimatization, the experimental animals were divided into four groups, comprising six rats each. Rats in the normal control group received a standard diet for a period of 30 days. Betaine group animals were orally administered with betaine [250 mg (dissolved in distilled water)/kg body weight/day] by intragastric intubation for a period of 30 days. In the isoprenaline group, rats were injected with isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight/day] i.p. for 2 days. In the betaine and isoprenaline group, animals were pretreated with betaine [250 mg/kg body weight/day] for 30 days before the induction of myocardial infarction as described for the isoprenaline group. Control animals (normal control group and betaine groups) were injected with physiological saline alone for 2 days. At the end of the experiment, i.e., 24 h after the last dose of isoprenaline, the experimental animals were killed by using chloroform anesthesia and blood was collected with anticoagulant for the separation of plasma. The heart tissue was dissected out immediately and washed with chilled physiological saline. Lysosomal fractions of the heart tissue were used for the determination of β -glucuronidase, β -glucosidase, β -galactosidase, acid phosphatase, and lipid peroxidation.

Biochemical assays

Lysosomal fractions from the hearts were isolated by the method of Plummer (1998). Immediately after killing the rats, hearts were removed and all the blood vessels and connective tissues were trimmed off. Hearts were washed free of blood in ice-cold sucrose, lightly blotted, and weighed. The hearts were cut into small fragments and homogenized in buffer containing 0.25 M sucrose and 1 mM ethylenediaminetetraacetic acid. The homogenates were centrifuged in a refrigerated centrifuge at 2,000 rpm for 10 min. The supernatants were transferred into test tubes. The pellets were resuspended in sucrose buffer and centrifuged again for 10 min at 2,000 rpm. The supernatants from each heart were pooled and centrifuged for 10 min at 7,400 rpm to produce the first lysosomal pellets. The supernatants were centrifuged again for 10 min at 11,400 rpm to produce the second lysosomal pellets. The lysosomal pellets were resuspended in sucrose and pelleted again. The lysosomal pellets were resuspended in about 2 mL of sucrose for use as the enzyme sources were stored on ice until required.

Assay of β -glucuronidase

β -Glucuronidase activity was measured by the method of Kawai and Anno (1971) using *p*-nitrophenol- β -glucuronide

as the substrate. The enzyme solution (0.2 mL) was added to 0.5 mL of substrate and 0.3 mL of sodium acetate buffer in a test tube, shaken gently, and incubated at 37°C for 1 h. Glycine-NaOH buffer (3.0 mL) was added for reaction termination, mixed, and read at 410 nm. The activity of β -glucuronidase was expressed as micromoles of *p*-nitrophenol liberated per hour per 100 mg protein.

Assay of β -glucosidase

β -Glucosidase was assayed according to the method of Conchie et al. (1967) based on the principle that β -glucosidase acts on *p*-nitrophenyl- β -D-glucopyranoside and liberates *p*-nitrophenol, which was measured at 410 nm in alkaline pH. The enzyme solution (0.2 mL) was added to 0.5 mL of substrate and 0.3 mL of citrate buffer in a test tube, shaken gently, and incubated at 37°C for 1 h. Glycine-NaOH buffer (3 mL) was added for reaction termination, mixed, and read at 410 nm using the Shimadzu UV-1601 spectrophotometer. The activity of β -glucosidase was expressed as micromoles of *p*-nitrophenol liberated per hour per 100 mg protein.

Assay of β -galactosidase

β -Galactosidase was assayed according to the method of Conchie et al. (1967) based on the principle that β -galactosidase acts on *p*-nitrophenyl- β -D-glucopyranoside and liberates *p*-nitrophenol, which was measured at 410 nm in alkaline pH. The enzyme solution (0.2 mL) was added to 0.5 mL of substrate and 0.3 mL of citrate buffer in a test tube, shaken gently, and incubated at 37°C for 1 h. Glycine-NaOH buffer (3 mL) was added for reaction termination, mixed, and read at 410 nm. The activity of β -galactosidase was expressed as micromoles of *p*-nitrophenol liberated per hour per 100 mg protein.

Assay of acid phosphatase

Acid phosphatase was assayed by the method of King (1965), using disodium phenyl phosphate as the substrate. The incubation mixture contained the following components in a final volume of 3.0: 1.5 mL of citrate buffer, 1.0 mL of substrate, 0.3 mL of distilled water, and the requisite amount of the enzyme source (0.2 mL plasma). The reaction mixture was incubated at 37°C for 15 min. The reaction was terminated by the addition of 1.0 mL of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent; 1.0 mL of 15% sodium carbonate solution was added and incubated for a further 10 min at 37°C. The blue color developed was read at 640 nm using a Shimadzu-

UV-1601 spectrophotometer against a blank. The standards were also treated similarly. The activity of the enzyme is expressed as micromoles of phenol liberated per hour per liter (plasma) or micromoles of phenol liberated per milligram protein (tissue).

Assay of lipid peroxidation

Lipid peroxidation was assayed by the method of Ohkawa et al. (1979) in which the malondialdehyde released served as the index of lipid peroxidation. 1,1,3,3-Tetra ethoxypropane malondialdehyde bis (diethyl acetal) was used as standard. To 0.2 mL of lysosomal suspension, 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid were added. The mixture was made up to 4.0 mL with water then heated in a water bath at 95.8°C for 60 min using a glass ball as a condenser. After cooling, 1.0 mL of water and 5 mL of *n*-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of lipid peroxides was expressed as nanomoles of malondialdehyde formed per milligram protein.

Statistical analysis

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

Results and discussion

Lysosomes are single membrane-bound subcellular organelles that are thought to be the chief degradatory compartments in the endosomal pathway. They house a diverse complement of hydrolases that function optimally at acidic

pH values. The pivotal role played by lysosomes in cell metabolism is manifested by the occurrence of at least 40 enzymopathies (Meikle et al. 1999) affecting the catabolism of neutral lipids, phospholipids, and glycolipids; complex carbohydrates; and proteins where indigestible macromolecular intermediates become stored inside the organelle, altering the homeostasis of the cell and leading to a disease state. Lysosomal membranes are reported to contain large amounts of glycoproteins, which play an important role in maintaining lysosomal structure and functions (Chen et al. 1985). Oxygen free radicals generated during ischemia, in addition to the direct myocardial damaging effect, may also be responsible for the cardiac damage through the release of lysosomal enzymes (Kalra and Prasad 1994). More recently, lysosomes have been found to be highly dynamic organelles involved in regulated secretion (Jaiswal et al. 2002) and repair of damaged plasma membrane (Reddy et al. 2001). In addition to the storage diseases, lysosomes have been implicated in a wide range of other human pathologies such as Alzheimer's disease (Pasternak et al. 2003; Pasternak et al. 2004), autoimmune diseases, and resistance to infectious disease, cancer, and drugs.

In the present study, the levels of lysosomal hydrolases (β -glucuronidase, β -galactosidase, β -glucosidase, and acid phosphatase) were significantly ($p<0.05$) higher in plasma of isoprenaline myocardial infarction-induced rats compared to that of normal control rats, indicating the severity of isoprenaline-induced necrotic damage to the myocardium (Table 1). A parallel decline in the activities of these hydrolytic enzymes in lysosomal fraction of the heart tissue was also observed (Table 2). The disintegration of myocardial membrane noticed in the present study may be due to the increased leakage of the lysosomal hydrolases from the enclosed sacs into the cytosol. This is in accordance with an earlier report (Ebenezar et al. 2003) that the cytosolic acid hydrolases liberated from lysosomes and from the sarcoplasmic reticulum induce the dysfunction and destruction of mitochondria, sarcolemma, and other organelles. Measurement of these hydrolytic enzymes in systemic circulation is frequently used as an index of

Table 1 Levels of β -glucuronidase, β -galactosidase, β -glucosidase, and acid phosphatase in the plasma of normal and experimental groups of rats

Parameters	Normal control group	Betaine group	Isoprenaline group	Betaine and isoprenaline group
β -Glucuronidase	9.80±0.69a	9.50±0.67a	16.20±1.15b	10.2±0.72a
β -Galactosidase	9.50±0.67a	9.00±0.63a	16.20±1.15b	9.20±0.65a
β -Glucosidase	7.50±0.53a	8.20±0.58a,b	15.10±1.07c	8.50±0.60b
Acid phosphatase	0.18±0.01a	0.15±0.01a	0.90±0.06b	0.25±0.02b

Betaine, 250 mg/kg body weight/day, p.o. for 30 days; isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days. Results are the mean±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different letter (a, b, c) differ significantly ($p<0.05$) with each other. β -Glucuronidase, β -glucosidase, and β -galactosidase were expressed as micromoles of *p*-nitrophenol liberated per hour per 100 mg protein. Acid phosphatase enzyme was expressed as micromoles of phenol liberated per hour per liter

Table 2 Levels of β -glucuronidase, β -galactosidase, β -glucosidase, acid phosphatase, and lipid peroxides in the heart lysosomes of normal and experimental group of rats

Parameters	Normal control group	Betaine group	Isoprenaline group	Betaine and isoprenaline group
β -Glucuronidase	30.90 $\pm$ 2.16a	29.50 $\pm$ 2.05a	17.50 $\pm$ 1.22b	26.50 $\pm$ 1.85c
β -Galactosidase	24.10 $\pm$ 1.68a	24.20 $\pm$ 1.69a	16.50 $\pm$ 1.15b	21.50 $\pm$ 1.50c
β -Glucosidase	21.20 $\pm$ 1.48a	21.50 $\pm$ 1.50a	13.60 $\pm$ 0.95b	20.20 $\pm$ 1.41a
Acid phosphatase	85.20 $\pm$ 5.96a,b	90.20 $\pm$ 6.31b	60.40 $\pm$ 4.22c	83.50 $\pm$ 5.84a
Lipid peroxides	0.79 $\pm$ 0.03a	0.72 $\pm$ 0.04b	2.18 $\pm$ 0.01c	0.94 $\pm$ 0.04d

Betaine, 250 mg/kg body weight/day, p.o. for 30 days; isoprenaline, 11 mg/100 g body weight/day, i.p. for 2 days. Results are the mean $\pm$ SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different letter (a, b, c, d) differ significantly ($p < 0.05$) with each other. β -Glucuronidase, β -glucosidase, and β -galactosidase were expressed as micromoles of *p*-nitrophenol liberated per hour per 100 mg protein. Acid phosphatase enzyme was expressed as micromoles of phenol liberated per milligram protein. Lipid peroxides was expressed as nanomoles of malondialdehyde released per milligram protein

lysosomal membrane integrity (Ravichandran et al. 1990). The higher levels in plasma and lower activities in lysosomal fractions of these enzymes, as observed in the present study, may be due either to the infiltration of inflammatory cells or due to isoprenaline-mediated lysosomal fragility by Ca^{2+} overload. A necrotic cell death can be triggered by a too strong lysosomal membrane permeabilization (Li et al. 2000) and oxygen free radicals are primarily responsible for the release of lysosomal hydrolases. It has also been suggested that abnormal release and activation of lysosomal enzymes during ischemia and other potentially lethal events may contribute to the tissue damage. Therefore, oxygen free radicals in addition to their direct myocardial damaging effect may also be responsible for cardiac damage through the release of lysosomal enzymes (Kalra et al. 1989).

Pretreatment with betaine preserved lysosomal integrity as evidenced by near normal levels of lysosomal hydrolases in heart tissue of betaine and isoprenaline group rats compared to isoprenaline-injected rats, indicating the cytoprotective effect of betaine. The antioxidant nature of betaine might have protected the lysosomal membrane from isoprenaline-induced reactive oxygen species and maintained the lysosomal membrane integrity. Since the release of lysosomal enzymes plays an important role in the progression of myocardial injury, maintaining the stability of the lysosomal membrane and hence preventing the release of its contents is a vital concept involved in the mechanism of cardioprotection. Methyl donors such as betaine, methionine, or folic acid prevented arsenite-induced cytotoxicity and reduced the oxidative stress and lysosomal damage without altering the functional integrity of the mitochondrial membrane and adenosine triphosphate depletion (Pourahmad et al. 2005). Natural antioxidant-rich diet such as table beet (*Beta vulgaris* var. *rubra*) contains important bioactive agents (betaine and polyphenols), which have a wide range of physiologic effects, and this has a positive effect on redox homeostasis during hepatic

ischemia–reperfusion in rats (Váli et al. 2007). Betaine is a potent protectant against bile acid-induced apoptosis in rats both in vivo and in vitro, and its antiapoptotic action largely resides on an inhibition of the proapoptotic mitochondrial pathway (Graf et al. 2002).

Lipid peroxidation levels observed in the heart lysosomes of isoprenaline group rats were significantly ($p < 0.05$) higher compared with the betaine and isoprenaline group rats (Table 2). This is consistent with an earlier study (Farvin et al. 2006) which also indicated that lipid peroxidation is a key factor in the pathogenesis of isoprenaline-induced myocardial infarction. The enhanced lipid peroxidation in heart lysosomes of isoprenaline-administered rats might have been due to the reduction in tissue antioxidant defense status, as reported earlier (Ganesan et al. 2008b).

In the present study, oral pretreatment with betaine significantly ($p < 0.05$) prevented the isoprenaline-induced lipid peroxidation in heart lysosomes of betaine and isoprenaline group rats at near normalcy. It probably did so by its antioxidant action (Craig 2004) against isoprenaline-induced lipid peroxidation (Rathore et al. 1998). Betaine is highly lipotropic and, when administered exogenously, can readily pass across the membrane lipid bilayer and inhibit lipid peroxidation in cellular membranes as a result of distinct biophysical interactions with the membrane lipid bilayer (Junnala et al. 1998). Betaine supplementation was effective in the prevention of lipopolysaccharide-induced necrotic damage in liver by inhibiting Kupffer cell activation and behaving as an antioxidant (Kim et al. 1998). Subsequent reports indicated that betaine had a potent reducing effect on the production of free radicals in rats exposed to cytotoxicity (Balkan et al. 2004; Kanbak et al. 2001). Feeding SH-generating substances such as methionine or nonenzymatic antioxidants such as glutathione have been reported to protect cellular and subcellular membranes from toxic free radical metabolites (Selvam and Kannabiran 1993). Betaine is involved in the synthesis of methionine, which serves as a major

supplier of cellular cysteine via the transsulfuration pathway for the synthesis of reduced glutathione that protects the cell from reactive metabolites and reactive oxygen species (Kim and Kim 2002).

In conclusion, the results of the present study indicated that the lysosomal hydrolases play an important role in isoprenaline-induced myocardial infarction. Prior oral administration of betaine proved to be more protective in reducing the extent of lysosomal membrane damage and preserve the lysosomal integrity in experimentally induced myocardial infarction in rats.

References

- Anandan R, Asha KK, Ammu K, Mathew S, Viswanathan Nair PG (2003) Effects of peroxidised PUFA on tissue defense system in experimentally induced myocardial infarction in rats. In: Surendran PK, Mathew PT, Thampuran N, Nambiar N, Joseph J, Boopendranath MR, Lakshmanan PT, Viswanathan Nair PG (eds) *Seafood safety*. Society of Fisheries Technologists, Cochin, India, pp 330–335
- Balkan J, Oztezcan S, Kucuk M, Cevikbas U, Kocak-Toker N, Uysal M (2004) The effect of betaine treatment on triglyceride levels and oxidative stress in the liver of ethanol-treated guinea pigs. *Exp Toxicol Pathol* 55:505–509. doi:10.1078/0940-2993-00347
- Chen JW, Murphy TL, Willingham MC, Patan I, August JT (1985) Identification of two lysosomal membrane glycoproteins. *J Cell Biol* 101:85–95. doi:10.1083/jcb.101.1.85
- Conchie J, Gelman AL, Levvy GA (1967) Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4 and C-6 specificity of beta glucosidase and beta galactosidase. *Biochem J* 103:609–615
- Craig S (2004) Betaine in human nutrition. *Am J Clin Nutr* 80:539–549
- Ebenezar KK, Sathish V, Devaki T (2003) Effect of L-arginine and L-lysine on lysosomal hydrolases and membrane bound phosphatases in experimentally induced myocardial infarction in rats. *Mol Cell Biochem* 247:163–169. doi:10.1023/A:1024115714236
- Farvin KHS, Anandan R, Kumar SHS, Shiny KS, Sankar TV, Thankappan TK (2004) Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. *Pharmacol Res* 50:231–236. doi:10.1016/j.phrs.2004.03.004
- Ganesan B, Rajesh R, Anandan R, Dhandapani N (2007a) Biochemical studies on the protective effect of betaine on mitochondrial function in experimentally induced myocardial infarction in rats. *J Health Sci* 53:671–681. doi:10.1248/jhs.53.671
- Ganesan B, Rajesh R, Anandan R, Dhandapani N (2007b) Protective effect of betaine on changes in the levels of protein, glycoproteins and amino acids in isoprenaline-induced myocardial infarction in rats. *African Journal of Biochemistry Research* 1:117–123
- Ganesan B, Anandan R, Rajesh R (2008a) Protective effect of betaine on changes in lipid profile, lipoproteins and fatty acid composition in experimentally induced myocardial infarction in Wistar rats. *Journal of Biomedical and Pharmaceutical Sciences* 2:65–69
- Ganesan B, Buddhan S, Anandan R, Sivakumar R, Anbin Ezhilan R (2008b) Antioxidant defense of betaine against isoprenaline-induced myocardial infarction in rats. *Mol Biol Rep* (in press)
- Graf D, Kurz AK, Reinehr R, Fischer R, Kircheis G, Häussinger D (2002) Prevention of bile acid-induced apoptosis by betaine in rat liver. *Hepatology* 36:829–839
- Gupta R, Gupta VP (1998) Lessons for prevention of coronary heart disease epidemiological study in western India. *Curr Sci* 74:1074–1077
- Hanson AD, Rathinasabapathi B, Rivoal J, Burnet M, Dillon MO, Gage DA (1994) Osmoprotective compounds in the plumbaginaceae: a natural experiment in metabolic engineering of stress tolerance. *Proc Natl Acad Sci U S A* 91:306–310. doi:10.1073/pnas.91.1.306
- Ithayarasi AP, Devi CSS (1997) Effect of alpha tocopherol on lipid peroxidation in isoproterenol induced myocardial infarction in rats. *Indian J Physiol Pharmacol* 41:369–376
- Jaiswal JK, Andrews NW, Simon SM (2002) Membrane proximal lysosomes are the major vesicles responsible for calcium-dependent exocytosis in nonsecretory cells. *J Cell Biol* 159:625–635. doi:10.1083/jcb.200208154
- Junnala M, Barak AJ, Beckenhauer HC, Rahko T (1998) Betaine reduces hepatic lipidosis induced by carbon tetrachloride in Sprague–Dawley rats. *Vet Hum Toxicol* 40:263–266
- Junnala M, Rahko T, Sukura A (2000) Reduction of carbon tetrachloride-induced hepatotoxic effect by oral administration of betaine in male Han–Wistar rats: a morphometric histological study. *Vet Pathol* 37:231–238. doi:10.1354/vp.37-3-231
- Kakreja RC, Hess ML (1992) The oxygen free radical system from equations through membrane–protein interactions to cardiovascular injury and protection. *Cardiovasc Res* 26:641–655. doi:10.1093/cvr/26.7.641
- Kalra J, Prasad K (1994) Oxygen free radicals and cardiac depression. *Clin Biochem* 27:163–168. doi:10.1016/0009-9120(94)90050-7
- Kalra J, Chaudhary AK, Prasad K (1989) Role of oxygen free radicals and pH on the release of cardiac lysosomal enzymes. *J Mol Cell Cardiol* 21:1125–1136. doi:10.1016/0022-2828(89)90690-1
- Kanbak G, Akyuz F, Inal M (2001) Preventive effect of betaine on ethanol-induced membrane lipid composition and membrane ATPases. *Arch Toxicol* 75:59–61. doi:10.1007/s002040000179
- Kawai Y, Anno K (1971) Mucopolysaccharides degrading enzymes from the liver of the squid *Ommastrephes solani pacificus*. I. Hyaluronidase. *Biochim Biophys Acta* 242:428–436
- Kim SK, Kim YC (2002) Attenuation of bacterial lipopolysaccharide-induced hepatotoxicity by betaine or taurine in rats. *Food Chem Toxicol* 40:545–549. doi:10.1016/S0278-6915(01)00102-8
- Kim SK, Kim YC, Kim YC (1998) Effects of singly administered betaine on hepatotoxicity of chloroform in mice. *Food Chem Toxicol* 36:655–661. doi:10.1016/S0278-6915(98)00024-6
- King J (1965) Lactate dehydrogenase n practical clinical enzymology. Van Nostrand, London, pp 83–93
- Krishnaswami S (1998) Observations on serial changes in coronary artery disease in Indians. *Curr Sci* 74:1064–1068
- Li W, Yuan X, Nordgren G, Dalen H, Dubowchik GM, Firestone RA, Brunk UT (2000) Induction of cell death by the lysosomotropic detergent MSDH. *FEBS Lett* 470:35–39. doi:10.1016/S0014-5793(00)01286-2
- Meikle PJ, Hopwood JJ, Clague AE, Carey WF (1999) Prevalence of lysosomal storage disorders. *J Am Med Assoc* 281:249–254. doi:10.1001/jama.281.3.249
- Moeckel GW, Shadman R, Fogel JM, Sadrzadeh SM (2002) Organic osmolytes betaine, sorbitol and inositol are potent inhibitors of erythrocyte membrane ATPases. *Life Sci* 71:2413–2424. doi:10.1016/S0024-3205(02)02035-0
- Nagle DG, Zhou YD (2006) Natural product-derived small molecule activators of hypoxia-inducible factor-1 (HIF-1). *Curr Pharm Des* 12:2673–2688. doi:10.2174/13816120677698783
- Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem* 95:351–358. doi:10.1016/0003-2697(79)90738-3
- Pasternak SH, Bagshaw RD, Guiral M, Zhang S, Ackerley CA, Pak BJ, Callahan JW, Mahuran DJ (2003) Presenilin-1, nicastrin,

- amyloid precursor protein, and γ -secretase activity are colocalized in the lysosomal membrane. *J Biol Chem* 278:26687–26694. doi:10.1074/jbc.M304009200
- Pasternak SH, Callahan JW, Mahuran DJ (2004) The role of the endosomal/lysosomal system in amyloid-beta production and the pathophysiology of Alzheimer's disease: reexamining the spatial paradox from a lysosomal perspective. *J Alzheimers Dis* 6:53–65
- Plummer DT (1998) An introduction to practical biochemistry, 3rd edn. McGraw-Hill, UK, pp 268–269
- Pourahmad J, Rabiei M, Jokar F, O'Brien PJ (2005) A comparison of hepatocyte cytotoxic mechanisms for chromate and arsenite. *Toxicology* 206:449–460. doi:10.1016/j.tox.2004.08.002
- Rathore N, John S, Kale M, Bhatnagar D (1998) Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. *Pharmacol Res* 38:297–303. doi:10.1006/phrs.1998.0365
- Ravichandran LV, Puvanakrishnan R, Joseph KT (1990) Alterations in the heart lysosomal stability in isoproterenol induced myocardial infarction in rats. *Biochem Int* 22:387–396
- Ravichandran LV, Puvanakrishnan R, Joseph KT (1991) Influence of isoproterenol induced myocardial infarction on certain glycohydrolases and cathepsins in rats. *Biochem Med Metab Biol* 45:6–15. doi:10.1016/0885-4505(91) 90003-4
- Reddy A, Caler EV, Andrews NW (2001) Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. *Cell* 106:157–169. doi:10.1016/S0092-8674(01) 00421-4
- Farvin KHS, Anandan R, Hari Senthil Kumar S, Shiny KS, Mathew S, Sankar TV, Viswanathan Nair PG (2006) Cardioprotective effect of squalene on lipid profile in isoprenaline-induced myocardial infarction in rats. *J Med Food* 9:531–536. doi:10.1089/jmf.2006.9.531
- Saunderson CL, Mackinlay J (1990) Changes in body weight, composition and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. *Br J Nutr* 63:339–349. doi:10.1079/BJN19900120
- Selvam R, Kannabiran K (1993) Induction of oxalate binding by dehydroascorbic acid feeding in rat kidney. *Nutr Res* 13:667–676. doi:10.1016/S0271-5317(05) 80559-7
- Todd GL, Cullan GE, Cullan GM (1980) Isoproterenol-induced myocardial necrosis and membrane permeability alteration in the isolated perfused rabbit heart. *Exp Mol Pathol* 33:43–54. doi:10.1016/0014-4800(80) 90006-4
- Váli L, Stefanovits-Bányai É, Szentmihályi K, Fébel H, Sárdi É, Lugasi A, Kocsis I, Blázovics A (2007) Liver-protecting effects of table beet (*Beta vulgaris* var. *rubra*) during ischemia–reperfusion. *Nutrition* 23:172–178. doi:10.1016/j.nut.2006.11.004
- Yusuf S, Reddy S, Ounpuu S, Anand S (2001) Global burden of cardiovascular diseases. Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. *Circulation* 104:2855–2864. doi:10.1161/hc4701.099488