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Preventive effect of S-allyl cysteine sulfoxide (alliin) on cardiac marker enzymes and lipids in isoproterenol-induced myocardial injury

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Abstract

The present study was designed to evaluate the preventive effect of S-allyl cysteine sulfoxide (SACS) in isoproterenol (ISO)-induced myocardial ischaemia in male Wistar rats. Rats were pretreated with SACS (40 and 80 mg kg^{-1} body-weight) for 5 weeks. After the treatment period, ISO (150 mg kg^{-1} body-weight) was administered subcutaneously to rats at intervals of 24 h for 2 days. The activities of creatine kinase, creatine kinase-MB, lactate dehydrogenase, aspartate transaminase and alanine transferase were significantly increased in serum and significantly decreased in the hearts of ISO-treated rats. Pretreatment with SACS decreased the activities of these enzymes significantly in serum and significantly increased the activities in heart in ISO-treated rats. The levels of cholesterol, triglycerides and free fatty acids increased in serum and heart, while the levels of phospholipids increased in serum and decreased in heart in ISO-treated rats. SACS pretreatment showed a significant effect on the lipids studied. The activity of 3-hydroxy 3-methyl glutaryl coenzyme A (HMG CoA) reductase was significantly increased and the activity of lecithin cholesterol acyl transferase (LCAT) was significantly reduced in ISO-induced rats. Oral pretreatment with SACS significantly decreased the activity of HMG CoA reductase and significantly increased the activity of LCAT in ISO-induced rats. The levels of plasma thiobarbituric acid reactive substances and hydroperoxides were increased in ISO-treated rats. Pretreatment with SACS significantly decreased the levels of lipidperoxides in ISO-treated rats. The effect at a dose of 80 mg kg^{-1} body-weight was more effective than at a dose of 40 mg kg^{-1} body-weight and brought back all the biochemical parameters to near normal levels. Thus our study shows that SACS has a lipid-lowering effect in ISO-induced rats. Our study may have clinical relevance.

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Introduction

Cardiovascular diseases have become the number one killer disease in many parts of the world. Myocardial infarction (MI) is the irreversible necrosis of heart muscle secondary to prolonged ischaemia. It usually results from an imbalance of oxygen supply and demand. The appearance of cardiac enzymes in the circulation generally indicates myocardial necrosis. An increased risk of coronary heart disease is associated with increased serum total cholesterol concentration and low density lipoprotein (LDL) and decreased high density lipoprotein (HDL) (Brown & Goldstein 1986; Castelli et al 1986; Grundy 1986). Traditional therapies for ischaemia are aimed at restoring the balance between oxygen delivery and the myocardial demand of oxygen (Mitra & Panja 2005). Isoproterenol (ISO), a β-adrenergic agonist, has been found to cause severe stress in myocardium, resulting in infarct-like necrosis of heart muscle with an increase in the levels of lipids in the myocardium (Wexler & Greenberg 1978). Free radical generation and lipid peroxidation could be involved in ISO-induced cardiac damage (Singal et al 1982). The pathophysiological changes following ISO administration are comparable to those taking place in human MI (Wexler 1978). The incidences of cardiovascular complications and MI among patients are very high and the reasons for this are still unclear (Bozdogan et al 2000).

Garlic (*Allium sativum* L.) has been used worldwide not only as a flavouring agent, but also as a traditional medicine since ancient times (Shimpo et al 2002). Garlic and compounds derived from it have been found to have many therapeutic effects. The main active ingredient in garlic is S-allyl cysteine sulfoxide (SACS), commonly called alliin. It is an organosulfur compound, constructed from an allyl group, a sulfoxide group and the amino acid cysteine. SACS is also present in cooked garlic, garlic vinegars and aged extracts. Whole garlic contains 6 to 14 mg of alliin g^{-1} fresh weight and 18 to 42 mg g^{-1} dry weight (Lawson 1998). Extensive literature surveys have shown that there are no available scientific reports on the effect of SACS on MI. In this study, we evaluated the preventive effect of SACS against ISO-induced MI in rats.

Materials and Methods

Drugs and chemicals

SACS was received as a gift from Wakunaga Pharmaceutical Co., Ltd, Hiroshima, Japan. ISO and digitonin were purchased from Sigma Chemical Company Inc. (St Louis, MO, USA). All other biochemicals used in this study were of analytical grade.

Animals

Male albino Wistar rats, body-weight ranging from 170 to 200 g, were purchased from Srivenkateswara Enterprises, Bangalore, Karnataka, India. Animals were housed in polypropylene cages with free access to food and water during the course of the experiment. The rats were fed on a standard pellet diet (Tamilnadu Veterinary and Animal Science University, Chennai, India). This study was approved by the Animal Ethical Committee of SASTRA-Deemed University, Thanjavur, India.

Induction of MI

MI was induced in rats by a single subcutaneous injection of ISO hydrochloride (150 mg kg^{-1}) dissolved in 2 mL normal saline at an interval of 24 h for 2 days (Venkatachalam et al 2002).

Experimental design

The animals were divided into six groups of eight rats each: Group I, normal untreated rats given 2 mL of saline orally daily for 35 days; Groups II and III, normal rats treated with SACS 2 mL (40 and 80 mg kg^{-1}, respectively) in saline by gastric intubation daily for 35 days; Group IV, rats subcutaneously injected with ISO in 2 mL of saline (150 mg kg^{-1}) once a day for 2 days; Groups V and VI, rats pretreated with SACS (40 mg kg^{-1} and 80 mg kg^{-1}, respectively) in 2 mL saline by gastric intubation daily for 35 days and then subcutaneously injected with ISO (150 mg kg^{-1}) once a day for 2 days.

Twenty-four hours after the last dose of ISO, blood was collected and the serum separated. Immediately after the sacrifice, the rats were dissected; the heart and liver were removed and washed in ice-cold saline. About 250 mg of the tissue was weighed and homogenized in chilled 0.1 M Tris HCl buffer in a Potter–Elvejhem Teflon homogenizer. The homogenate was used for the estimation of various biochemical parameters.

Assay of cardiac marker enzymes

The activity of creatine kinase (CK) was assayed by the method of Okinaka et al (1961). Creatine kinase-MB (CK-MB) activity was assayed by the immuno inhibition method of Stein (1998) using a reagent kit (Euro Diagnostic Systems Pvt. Ltd, Okkiyam Thuraipakkam, Chennai, Tamilnadu, India). The activity of lactate dehydrogenase (LDH) was assayed by the method of King (1965). The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were assayed by the method of Reitman & Frankel (1957) using reagent kits (Span Diagnostics Ltd, Udhna, Gujarat, India).

Estimation of lipid profile in serum and heart

The lipid extraction of heart tissue was carried out by the method of Folch et al (1957). The levels of total, free and ester cholesterol, free fatty acids and phospholipids in serum and heart were estimated according to the methods of Zlatkis et al (1953), Varley et al (1991), Falholt et al (1973) and Zilversmit & Davis (1950), respectively. The concentration of triglycerides in serum and heart was measured by the GOP-POD method using a reagent kit (Medsource Ozone Biomedical Pvt. Ltd, Sant Nager, East of Kailash, New Delhi, India). Serum HDL was determined using a kit (Product No. 7221, Sigma Diagnostics Pvt. Ltd, Baroda, India). LDL and very low density lipoprotein (VLDL) fractions were calculated as VLDL = triglyceride/5 and LDL = total cholesterol – (HDL cholesterol + VLDL cholesterol), respectively. Tissue protein for enzyme activity was determined by the method of Lowry et al (1951).

Assay of HMG CoA reductase, LCAT and estimation of lipid peroxides

The activity of 3-hydroxy 3-methyl glutaryl coenzyme A (HMG CoA) reductase was assayed by measuring the ratio of HMG CoA to mevalonate by the method of Venugopala Rao & Ramakrishnan (1975), and the activity of lecithin cholesterol acyl transferase (LCAT) was assayed by the ratio of ester cholesterol to unesterified cholesterol. Thiobarbituric acid reactive substances (TBARS) in plasma were estimated by the

method of Yagi (1987). The lipid hydroperoxides (HP) were estimated by the method of Jiang et al (1992).

Statistical analysis

Statistical analysis was performed using the SPSS software package, version 10.0. The values were analysed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) (Duncan 1957). All the results were expressed as mean ± s.d. for eight rats in each group. $P < 0.05$ was considered to be significant.

Results

A significant ($P < 0.05$) increase in heart weight was observed in the ISO-treated rats (866.87 + 67.23 mg) compared with normal control rats (768.25 + 34.55 mg). Normal rats treated with SACS (40 and 80 mg kg^{-1}) for 35 days did not show any significant (749.12 + 50.75 mg and 723.25 + 31.07 mg) effects. Pretreatment with SACS (40 and 80 mg kg^{-1}) for 35 days significantly ($P < 0.05$) decreased heart weight (811.00 + 32.19 mg and 775.50 + 67.23 mg) in ISO-treated rats.

Table 1 shows the effect of SACS on the activities of CK, CK-MB, LDH, AST and ALT in the serum and heart of normal and ISO-induced rats. ISO-administered rats showed a significant ($P < 0.05$) increase in the activities of these enzymes in serum and a significant decrease in heart compared to normal rats. Pretreatment with SACS (40 and 80 mg kg^{-1}) for 35 days significantly ($P < 0.05$) decreased CK, CK-MB, LDH, AST and ALT activities in serum and increased activities in heart in ISO-treated rats.

Table 2 represents the total cholesterol, HDL, LDL, VLDL cholesterol, free and ester cholesterol in normal and ISO-induced rats. A significant ($P < 0.05$) increase in the serum LDL and VLDL fractions, along with a significant decrease in the HDL fraction, was observed in ISO-induced rats compared to normal rats. ISO-induced rats also showed a significant ($P < 0.05$) increase in total, free and ester cholesterol in the heart when compared with normal rats. Pretreatment with SACS (40 and 80 mg kg^{-1}) for 35 days significantly ($P < 0.05$) decreased the total, ester and free cholesterol, LDL and VLDL fractions and significantly ($P < 0.05$) increased the HDL fraction in ISO-treated rats.

Table 3 shows the effect of SACS on serum and heart triglycerides, free fatty acids and phospholipids in normal and ISO-treated rats. ISO-induced rats showed a significant ($P < 0.05$) increase in triglycerides, free fatty acids in serum and heart, and phospholipids in serum, and a significant ($P < 0.05$) decrease in phospholipids in heart compared to normal rats. Oral pretreatment with SACS (40 and 80 mg kg^{-1}) for 35 days significantly ($P < 0.05$) decreased the concentration of triglycerides, free fatty acids in serum and heart, and phospholipids in serum, and increased the phospholipids in heart in ISO-induced rats.

Table 4 shows the activities of HMG CoA reductase and LCAT, and the levels of TBARS and HP in normal and ISO-treated rats. ISO-induced rats showed a significant ($P < 0.05$) increase in the activity of HMG

Table 1 Effect of SACS on the activities of CK, CK-MB, LDH, ALT and AST in serum and heart in normal and ISO-treated rats

Group		Group I	Group II	Group III	Group IV	Group V	Group VI
CK	Serum (IU L^{-1})	283.20 ± 2.5^{a}	283.53 ± 3.61^{a}	280.40 ± 3.18^{a}	424.82 ± 3.64^{b}	353.36 ± 4.92^{c}	311.83 ± 6.24^{d}
	Heart (μmoles of phosphorus liberated min^{-1} mg^{-1} protein)	16.03 ± 0.34^{a}	16.25 ± 0.33^{a}	16.40 ± 0.56^{a}	9.67 ± 0.46^{b}	11.26 ± 0.61^{c}	15.32 ± 0.38^{d}
CK-MB	Serum (IU L^{-1})	16.07 ± 0.28^{a}	16.24 ± 0.40^{a}	16.17 ± 0.38^{a}	35.50 ± 0.28^{b}	27.98 ± 0.60^{c}	20.00 ± 0.65^{d}
	Heart (U mg^{-1} wet tissue)	22.85 ± 0.53^{a}	22.56 ± 0.53^{a}	23.16 ± 1.49^{a}	19.43 ± 0.72^{b}	20.61 ± 0.89^{c}	23.50 ± 0.70^{a}
LDH	Serum (IU L^{-1})	90.71 ± 0.57^{a}	90.40 ± 2.42^{a}	89.86 ± 4.47^{a}	131.37 ± 3.42^{b}	115.56 ± 2.04^{c}	101.92 ± 3.22^{d}
	Heart (nmoles of pyruvate liberated min^{-1} mg^{-1} protein)	110.50 ± 3.00^{a}	109.87 ± 3.40^{a}	109.76 ± 3.28^{a}	72.52 ± 2.70^{b}	81.26 ± 3.27^{c}	85.51 ± 6.65^{d}
AST	Serum (IU L^{-1})	30.33 ± 1.32^{a}	29.55 ± 1.86^{a}	31.21 ± 1.32^{a}	44.30 ± 4.22^{b}	42.35 ± 2.7^{b}	37.06 ± 1.90^{c}
	Heart (nmoles of pyruvate liberated min^{-1} mg^{-1} protein)	30.23 ± 1.28^{a}	29.40 ± 1.00^{a}	29.07 ± 1.03^{a}	25.35 ± 1.70^{b}	32.02 ± 1.92^{c}	31.23 ± 1.28^{dc}
ALT	Serum (IU L^{-1})	10.07 ± 0.57^{a}	10.15 ± 0.60^{a}	10.26 ± 0.73^{a}	25.1 ± 0.35^{b}	20.12 ± 0.36^{c}	12.13 ± 0.38^{d}
	Heart (nmoles of pyruvate liberated min^{-1} mg^{-1} protein)	27.97 ± 0.38^{a}	28.07 ± 0.52^{a}	28.31 ± 0.64^{a}	16.03 ± 0.38^{b}	21.13 ± 0.50^{c}	23.17 ± 0.40^{d}

Each value is mean ± s.d. for eight samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 2 Effect of SACS on total, ester and free cholesterol and lipoproteins in normal and ISO-treated rats

Group		Group I	Group II	Group III	Group IV	Group V	Group VI
Total cholesterol	Serum (mg dL^{-1})	84.61 ± 5.78^{a}	82.48 ± 6.7^{a}	80.26 ± 8.8^{a}	136.50 ± 9.27^{b}	111.95 ± 11.15^{c}	94.87 ± 2.83^{d}
	Heart (mg g^{-1} wet weight)	7.72 ± 0.31^{ad}	7.23 ± 0.68^{a}	7.20 ± 0.41^{a}	15.48 ± 1.20^{b}	11.62 ± 0.81^{c}	8.43 ± 0.67^{d}
HDL cholesterol	Serum (mg dL^{-1})	34.25 ± 0.59^{a}	34.59 ± 0.63^{a}	34.16 ± 0.43^{a}	18.46 ± 0.72^{b}	21.62 ± 0.77^{c}	28.28 ± 0.58^{d}
LDL cholesterol	Serum (mg dL^{-1})	41.46 ± 3.90^{a}	40.12 ± 0.96^{a}	36.72 ± 1.11^{a}	106.90 ± 9.60^{b}	83.93 ± 7.51^{c}	49.76 ± 1.95^{d}
VLDL cholesterol	Serum (mg dL^{-1})	8.9 ± 0.67^{ad}	8.7 ± 0.82^{a}	8.8 ± 0.46^{ad}	11.12 ± 0.60^{b}	8.33 ± 0.30^{c}	6.72 ± 0.33^{d}
Ester cholesterol	Serum (mg dL^{-1})	58.32 ± 1.20^{a}	57.67 ± 1.8^{a}	56.67 ± 2.1^{a}	77.75 ± 1.00^{b}	62.14 ± 1.64^{c}	65.09 ± 1.7^{d}
	Heart (mg g^{-1} wet weight)	4.09 ± 0.29^{ad}	3.82 ± 0.24^{a}	3.81 ± 0.29^{a}	7.98 ± 0.40^{b}	6.25 ± 0.42^{c}	5.86 ± 0.50^{d}
Free cholesterol	Serum (mg dL^{-1})	26.82 ± 1.74^{a}	26.63 ± 0.92^{a}	26.09 ± 0.73^{a}	53.07 ± 0.96^{b}	41.92 ± 0.99^{c}	28.35 ± 1.85^{d}
	Heart (mg g^{-1} wet weight)	3.27 ± 0.11^{a}	3.12 ± 0.26^{a}	3.30 ± 0.03^{a}	6.28 ± 0.12^{b}	5.31 ± 0.36^{c}	3.21 ± 0.04^{a}

Each value is mean ± s.d. for eight samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 3 Effect of SACS on serum and heart triglycerides, free fatty acids and phospholipids in normal and ISO-treated rats

Group		Group I	Group II	Group III	Group IV	Group V	Group VI
Triglycerides	Serum (mg dL^{-1})	44.06 ± 0.41^{a}	43.06 ± 0.38^{a}	42.91 ± 1.11^{a}	55.63 ± 1.61^{b}	41.36 ± 2.89^{c}	33.50 ± 1.06^{d}
	Heart (mg g^{-1} wet weight)	4.38 ± 0.15^{a}	4.36 ± 0.16^{a}	4.33 ± 0.17^{a}	6.49 ± 0.21^{b}	4.91 ± 0.4^{c}	4.48 ± 0.18^{d}
Free fatty acids	Serum (mg dL^{-1})	28.22 ± 1.44^{a}	27.31 ± 1.65^{a}	27.63 ± 1.78^{a}	42.56 ± 2.99^{b}	37.23 ± 1.80^{c}	32.48 ± 1.36^{d}
	Heart (mg g^{-1} wet weight)	0.51 ± 0.02^{a}	0.49 ± 0.02^{a}	0.49 ± 0.01^{a}	0.61 ± 0.03^{b}	0.59 ± 0.01^{b}	0.55 ± 0.03^{c}
Phospholipids	Serum (mg dL^{-1})	76.42 ± 2.1^{a}	76.00 ± 2.86^{a}	75.46 ± 2.57^{a}	98.67 ± 1.99^{b}	92.45 ± 1.82^{c}	84.97 ± 1.01^{d}
	Heart (mg g^{-1} wet weight)	31.10 ± 0.63^{a}	31.53 ± 0.56^{a}	30.68 ± 0.45^{a}	25.23 ± 0.52^{b}	27.95 ± 0.43^{c}	29.70 ± 1.03^{d}

Each value is mean ± s.d. for eight samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

Table 4 Effect of SACS on the activities of HMG CoA reductase and LCAT on heart and liver and lipid peroxides in plasma in normal and ISO-treated rats

Group	HMG CoA reductase*		LCAT (values expressed as difference in esterified/unesterified cholesterol)		TBARS (nmol mL^{-1})	HP (values $\times 10^{-5}$ mM dL^{-1})
	Heart	Liver	Heart	Liver	Plasma	Plasma
Group I	2.03 ± 0.04^{a}	2.34 ± 0.05^{a}	8.93 ± 0.02^{a}	10.34 ± 0.49^{ad}	3.17 ± 0.02^{a}	9.43 ± 0.04^{a}
Group II	1.99 ± 0.06^{a}	2.31 ± 0.06^{a}	8.97 ± 0.04^{a}	11.10 ± 0.95^{a}	3.15 ± 0.03^{a}	9.41 ± 0.04^{a}
Group III	1.98 ± 0.02^{a}	2.27 ± 0.07^{a}	9.03 ± 0.14^{a}	11.07 ± 0.27^{a}	3.15 ± 0.03^{a}	9.39 ± 0.04^{a}
Group IV	2.03 ± 0.04^{a}	3.50 ± 0.31^{b}	7.01 ± 0.03^{b}	8.21 ± 0.41^{b}	7.58 ± 0.20^{b}	15.81 ± 0.08^{b}
Group V	2.02 ± 0.04^{a}	2.98 ± 0.06^{c}	9.13 ± 0.35^{a}	8.97 ± 0.43^{c}	5.70 ± 0.29^{c}	12.51 ± 0.30^{c}
Group VI	2.03 ± 0.04^{a}	2.64 ± 0.05^{d}	10.12 ± 0.32^{c}	10.11 ± 0.32^{d}	3.80 ± 0.02^{d}	10.37 ± 0.46^{d}

*HMG CoA reductase is expressed as ratio of 3-hydroxyl 3-methylglutaryl coenzyme A to mevalonate. Each value is mean ± s.d. for eight samples in each group. Values not sharing a common superscript (a, b, c, d) differ significantly at $P < 0.05$ (DMRT).

CoA reductase in liver compared to normal rats. HMG CoA reductase activity in the heart did not show any significant change in ISO-treated rats. The activity of LCAT was depressed significantly ($P < 0.05$) in both heart and liver in ISO-induced rats compared to normal rats. ISO-treated rats also showed an increase in the levels of TBARS and HP in plasma. Pretreatment with SACS (40 and 80 mg kg^{-1}) for 35 days significantly ($P < 0.05$) decreased the activity of HMG CoA reductase in liver and significantly ($P < 0.05$) increased the activity of LCAT in heart and liver. SACS also significantly ($P < 0.05$) decreased plasma lipid peroxides in ISO-treated rats.

Discussion

Garlic is reported to have beneficial effects on the risk factors associated with cardiovascular disease, including normalization of plasma lipid levels (Banerjee & Maulik 2002; Brace 2002). Confirming these reports, previous studies with rabbits, rats and humans treated with garlic have also reported modulation of the lipids in plasma caused by a decrease in the concentration of plasma cholesterol and triglycerides and markers of cholesterol synthesis and absorption (Chi et al 1982; Eilat et al 1995; Bordia et al 1997; Augusti et al 2001; Brace 2002). Other studies with cultured rat hepatocytes have suggested that the inhibition of cholesterol synthesis at least partly explains the hypolipidaemic effect of garlic (Gebhardt 1993; Yeh & Liu 2001). Recently Santo et al (2004) reported that well-characterized garlic-derived materials are not hypolipidaemic in APOE*3-Leiden transgenic mice. This contradiction may result from the experimental design and/or the animal model. Feeding of garlic powder to rats for 11 days had a protective effect on ISO-induced myocardial damage (Ciplea & Richter 1988). Preliminary studies have shown that SACS exhibits anticholesterolaemic and hypolipidaemic effects (Nakamura & Ishikawa 1971; Sheela & Augusti 1992; Wilcox et al 1992). SACS also exhibits an antioxidant effect in rats given an atherogenic diet (Bopanna et al 1998). In this study, we report the preventive effect of SACS on cardiac enzymes, lipids, lipoproteins and enzymes associated with lipid metabolism by virtue of its hypolipidaemic, antilipoperoxidative and antioxidant properties in ISO-induced rats.

Our study shows that SACS protects the heart from ISO-induced MI. The marker enzymes of MI such as CK, CK-MB, LDH, AST and ALT are elevated in serum and decreased in heart. When CK, CK-MB, LDH, AST and ALT in heart are damaged due to deficiency of oxygen, the cell membrane becomes ruptured, which leads to leakage of the enzymes into the blood (Mathew et al 1985). Oral pretreatment of SACS in ISO-treated rats brings back the activities of these enzymes to near normal. This might be due to the protective effect of SACS on the heart, which reduces the extent of cardiac damage induced by ISO and thus restricts the leakage of these enzymes from myocardium.

We noted an increase in the weight of the heart in ISO-induced rats. Nirmala & Puvanakrishnan (1994) reported that the increase in heart weight in ISO-induced rats might be due to the increased water content, oedematous intramuscular space and extensive cardiac muscle fibre followed by invasion of the damage tissues by inflammatory cells. Oral pretreatment with SACS decreases heart weight in ISO-treated rats.

We observed an increase in the levels of total, free and ester cholesterol in the serum of ISO-induced rats. Increased levels of serum LDL and VLDL fractions, along with a decrease in serum HDL cholesterol, are also observed in ISO-induced rats. Increased levels of circulating cholesterol and its accumulation in myocardium are well associated with cardiovascular damage (Manjula et al 1992). Miller & Miller (1975) reported that increased levels of LDL cholesterol have a positive relation and increased levels of HDL cholesterol have a negative relation with MI. It has been shown that HDL is inversely related to body cholesterol. HDL inhibits the uptake of LDL by the arterial wall and also facilitates the transport of cholesterol from peripheral tissue to the liver, where it is catabolized and excreted out of the body (Carlson & Bottiger 1972). Oral pretreatment with SACS in ISO-induced rats decreases total, ester and free cholesterol. SACS pretreatment also increases the serum HDL fraction of ISO-induced rats.

Hypertriglyceridaemia is also associated with MI. The observed hypertriglyceridaemia in ISO-treated rats might be due to a decrease in lipoprotein lipase activity in heart, resulting in a decreased uptake of triglycerides from the circulation (Sheela Sasikumar & Devi 2001). In ISO-induced Wistar rats given pretreatment with SACS there is a reduction in triglycerides in serum and heart.

ISO administration also resulted in increased levels of free fatty acids and phospholipids. Chein et al (1980) reported an increased peroxidation of membrane phospholipids released from free fatty acids by the action of phospholipase A_2. They also stated that Ca^{2+} ions induce phospholipase A_2. Accelerated membrane phospholipid degradation results in cell injury and cell death (Chein et al 1978). Thus, the observed increase in the concentration of free fatty acids could have been due to the indirect effect of the calcium level, which was reported to be altered in ISO-induced rats (Shen & Jennings 1972). Pretreatment with SACS maintains phospholipids and free fatty acids at near-normal levels in ISO-treated rats.

We also noted an increase in the activity of HMG CoA reductase in liver in ISO-induced rats. The activity of HMG CoA reductase in heart did not show any significant change in ISO-treated rats. Similar results have been reported in ISO-treated rats (Kurian et al 2005). Oral pretreatment with SACS significantly reduces the activity of HMG CoA reductase in liver. The hypocholesterolaemic actions of SACS are related to both the inhibition of liver cholesterol biosynthesis and depletion of the cholesterol pool.

We noted a decrease in the activity of LCAT in heart and liver. This enzyme esterifies the cholesterol, which then becomes non-polar and moves into the core of the HDL, allowing the surface of the particle to accept more cholesterol. ISO-treated rats depressed the LCAT activity in heart and liver, thus the esterification of the cholesterol is

inhibited, which leads to a high concentration of serum lipids and lipoproteins, which result in high risk of atherosclerosis and coronary heart disease. That SACS increases HDL levels as described earlier is further evidenced by its prominent LCAT activity in heart and liver, which increases the concentration of good cholesterol (HDL) in ISO-induced rats.

We noticed an increase in the plasma TBARS and HP in ISO-treated rats. This showed enhanced lipid peroxidation, leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of free radicals. The increased levels of TBARS and HP also injure blood vessels, causing increased adherence and aggregation of platelets to the injured sites, leading to increased accumulation of lipids (Jeyalakshmi & Niranjali 2004). SACS administration prevents the accumulation of lipid-peroxides in ISO-treated rats. This effect might be due to the anti-lipoperoxidative property of SACS. In our laboratory we have also observed that SACS increases the activities of superoxide dismutase, catalase, glutathione peroxidase and the concentration of glutathione in myocardium in ISO-induced rats. This illustrates the antioxidant effect of SACS (data not shown).

Conclusion

Our results show that SACS protects the myocardium against the accumulation of lipids in ISO-induced rats. This might be due to the antioxidant effect of SACS. SACS scavenges free radicals and indirectly helps to decrease lipids by reducing or inhibiting the lipid peroxidation process. In this context, allicin, another constituent of garlic, also exhibits a lipid-lowering effect (Augusti & Mathew 1974). However, further studies are necessary to find out the exact mechanism of action of the lipid-lowering effect of SACS. The results from this study may have clinical relevance.

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