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Original article

Protective effect of betaine on protein, glycoproteins and amino acids in isoprenaline-induced myocardial infarction in albino rats

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ABSTRACT

Myocardial infarction is emerging as a foremost public health concern in most parts of the world even in developing countries still afflicted by infectious diseases, under nutrition and other illnesses related to poverty. There has been increasing recognition that certain natural substances have the potential to reduce the detrimental effect of a number of cardiovascular risk factors. In the present study, we have investigated the protective effect of betaine administration on changes in the levels of protein, glycoproteins and amino acids was studied in isoprenaline-induced myocardial infarction in rats as an animal model of myocardial infarction in man. Oral pre-treatment with betaine significantly attenuated ($P < 0.01$) the isoprenaline-induced rise in the levels of troponin-T and creatine phosphokinase [CPK]. Oral supplementation of betaine also significantly ($P < 0.01$) counteracted the isoprenaline-induced alterations in the levels of amino acids [taurine, aspartate, glutamate, arginine, hydroxy proline and homocysteine], protein content, glycoprotein components [hexose and hexosamine] and lipid peroxidation in the heart tissue and maintained their levels comparable to that of control animals. The results indicated that the overall cardioprotective effect of betaine was probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action or to a counteraction of free radicals by its antioxidant property.

1. Introduction

Myocardial infarction (MI) is one of the most widespread manifestations of cardiovascular disease. The morbidity and mortality due to myocardial infarction has reached epidemic proportion in the world, accounting for 16.7 million deaths/year worldwide (WHO, 2004). World Health Organization (WHO) envisages that heart disease and stroke will become the leading cause of both death and disability all over the world by 2020, with the number of fatalities projected to increase more than 20 million a year and to more than 24 million by 2030. In India, myocardial infarction typically occurs 10 to 15 years earlier than in the west. Low-density lipoprotein (LDL) is the major cholesterol carrying lipoprotein in plasma and is the causal agent in many forms of heart diseases [1]. Although major advances have been made in the treatment of cardiovascular diseases, myocardial infarction remains an important public health concern in many developing countries and will soon attain that status in several others. Epidemiological studies [3] have endowed with compelling evidence that occurrence of myocardial infarction is largely preventable.

Natural products have been the starting point for the discovery of many important modern drugs. This fact has led to biochemical and pharmacological investigations and general biological screening programs for natural products all over the world. Betaine is found in microorganisms, plants and animals and is a significant component of many foods, including wheat, shellfish, spinach, and sugar beets [4]. Betaine, also known as trimethylglycine or glycine betaine, is a quaternary amine, closely related to the amino acid, glycine. It is hypothesized that betaine is non-perturbing to cellular metabolism, highly compatible with enzyme functions, and stabilizes cellular metabolic function under different kinds of stress in various organisms and animal tissues [5]. Reports by Stekol et al. [6] indicated that administration of betaine exerted significant role within tissue as a methyl donor, which in turn may be used for the synthesis of methionine, carnitine, phosphatidylcholine, creatine and these substances play a key role in protein and energy metabolism in the myocardium.

Betaine has been reported to play a role in reducing blood levels of homocysteine, a toxic breakdown product of amino acid metabolism that is believed to promote atherosclerosis and

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osteoporosis. Betaine is now attracting increased research attention not only because of potential effects on lipid metabolism, but also re-partitioning agent and modulator of protein metabolism [7]. Though the beneficial properties of betaine are promising and well studied in hepatotoxicity, the protective effects of betaine on protein metabolism in experimentally induced myocardial infarction condition have not yet been explored.

Isoprenaline (ISO) is a synthetic β-adrenergic agonist that causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle [8]. Isoprenaline-induced myocardial infarction serves as a well-standardized model to study the beneficial effects of many drugs and cardiac functions [9]. Isoprenaline-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membranes [10]. It 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 [11].

In the present study, attempt has been made to assess the protective effects of betaine administration against isoprenaline-induced myocardial infarction in rats with respect to changes in the levels of creatine phosphokinase CPK, protein, glycoproteins, amino acids and lipid peroxidation.

2. Materials and methods

2.1. Procurement of chemicals and experimental animals

Amino acids, isoprenaline (isoproterenol) and betaine used in this study were obtained from M/s. Sigma Chemical Company, Saint-Louis. MO, USA. All the other chemicals used were of analytical grade. Wistar strain male albino rats, with weight range of 150–180 g, were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions. The prevailing environmental temperature was 28 ± 2 °C, relative humidity of 60–70% with 12-h light/dark cycle. The animals were allowed a standard diet [M/s Krish Feeds, Bangalore, India] and water *ad libitum*. Diet contained 56.2% carbohydrate, 22% crude protein, 7.5% ash, 4.2% crude oil, 3% crude fibre, 2.5% glucose, 1.8% vitamin, 1.4% sand silica, 0.8% calcium, 0.6% phosphorus and provided metabolizable energy of 3600 kcal. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.2. Induction of myocardial infarction and experimental procedure

The myocardial infarction was induced in experimental rats by intraperitoneal injection with isoprenaline at 11 mg/100 g body weight in physiological saline for 2 days [12]. Five days after acclimatization, the experimental animals were divided into four groups, comprising six rats each:

- group I (normal control) received standard diet for a period of 30 days;
- group II 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;
- group III, rats were injected with isoprenaline [11 mg (dissolved in physiological saline)/100 g body weight/day], for 2 days for the induction of myocardial infarction;
- group IV, the animals were pre-treated with betaine [250 mg/kg body weight/day] for 30 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 experiment, i.e. 24 h after the last dose of isoprenaline, the experimental animals were killed and blood was collected with anticoagulant (EDTA) for separation of plasma. The heart tissue was excised immediately and washed with chilled isotonic saline. Troponin-T content in plasma was determined by electrochemiluminescence immunoassay (ECLIA) using Modular Analytics E170 (Elecsys module) immunoassay analyzer. Homocysteine (tHcy) concentration in plasma was assayed by using Microtiter Plate Assay package (Diazyme Laboratories). The levels of taurine, asparate, glutamate, arginine and hydroxy proline in the heart tissue were determined according to the method of Ishida et al. [13] using Shimadzu LC 10 AS Amino Acid Analyzer. The protein content was estimated by the method of Lowry et al. [14]. The glycoprotein components, hexose and hexosamine, were determined by the methods described by [15,16], respectively. Lipid peroxidation (LPO) in the heart tissue was assayed by the method of [17] in which the malondialdehyde (MDA) released served as the index of LPO.

2.3. Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 and the data obtained were expressed as mean ± SD. Multiple comparisons of the means were separated using the Duncan Multiple Range Test at 5% probability.

3. Results and discussion

Myocardial infarction is one of the most significant and persistent public health problems in both developed and developing world. A better understanding of the processes involved in myocardial injury has stimulated the search for new drugs, which could limit the myocardial damage. The focus of the study was to evaluate the protective effects of betaine on changes in the levels of protein, glycoproteins and amino acids in isoprenaline-induced myocardial infarction in rats.

There was a significant increase ($P < 0.01$) in the troponin-T plasma concentration for rats injected with isoprenaline (i.e. group III) when compared to rats in the control group I. This indicate a damage to the contractile protein, which is responsible for the release of structurally bound troponin-T used as a marker for the detection of cardiac injury in laboratory animals [18]. Our findings confirm the same pattern, and revealed a significant increase in the level of troponin-T in plasma of group III isoprenaline-administered rats compared to group I control animals. Reports by Robertson et al. indicated that phosphorylation of troponins reduced myofilament sensitivity to Ca^{2+} ions, thereby contributing to higher rate of relaxation during β-adrenergic stimulation (Fig. 1). Troponins are regulatory proteins essential for contraction and relaxation processes in myocardium.

In the present study, administration of betaine significantly prevented the isoprenaline-induced release of troponin-T from myocardium into the blood stream, thereby demonstrating its protective action on the cell membrane. This perhaps is possible through the maintenance of the delicate balance of tonicity in cells in the myocardium. Betaine plays a major role in cell volume regulation by modulating the elasticity of plasma membrane [20]. Cell volume affects the most basic processes of cell function, and as such, it exerts an important role in the onset, severity, and outcome of myocardial infarction. Studies by Wettstein et al. [21] have shown that betaine can overt severe osmolar changes associated with possible cell death.

Recent prospective investigations [22] revealed that even mild hyper-homocysteinemia is associated with an increased risk of