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Preventive effects of N-acetyl cysteine on lipids, lipoproteins and myocardial infarct size in isoproterenol induced myocardial infarcted rats: An in vivo and in vitro study

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ABSTRACT

The present study evaluated the preventive effects of N-acetyl cysteine in isoproterenol induced myocardial infarcted rats. Rats were pretreated with N-acetyl cysteine (10 mg/kg body weight) daily for a period of 14 days. After pretreatment, rats were injected with isoproterenol (100 mg/kg body weight) at an interval of 24 h for two days to induce myocardial infarction. Isoproterenol induced myocardial infarction was indicated by increased activity of creatine kinase-MB and levels of cardiac troponins in the serum. The weight of heart and the levels of serum and heart cholesterol, triglycerides, free fatty acids were increased in isoproterenol induced myocardial infarcted rats. Isoproterenol also increased the levels of serum low density and very low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol. It enhanced the activity of liver 3-hydroxy-3 methyl glutaryl-Coenzyme-A reductase and the levels of lipid peroxidation products. Pretreatment with N-acetyl cysteine showed significant preventive effects in all the biochemical parameters studied in myocardial infarcted rats. Also, N-acetyl cysteine reduced myocardial infarct size. Histopathological findings of N-acetyl cysteine pretreated myocardial infarcted heart correlated with these biochemical findings. The *in vitro* study confirmed the strong antioxidant action of N-acetyl cysteine. Thus, the present study revealed that N-acetyl cysteine prevented increased heart weight, accumulation of lipids, altered levels of lipoproteins thereby reducing myocardial infarct size due to its antilipidemic and antioxidant effects in isoproterenol induced myocardial infarcted rats. This study may have a significant impact on myocardial infarction.

1. Introduction

Myocardial infarction is the most lethal manifestation of cardiovascular disease. It is the acute condition of necrosis of myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. The animal model of myocardial infarction plays an important role in the prevention, diagnosis and therapy of human myocardial infarction (Wang et al., 2006). Isoproterenol, a synthetic catecholamine causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle (Sushamakumari et al., 1989).

Isoproterenol increases lipids such as total cholesterol, triglycerides, free fatty acids and phospholipids in the circulation (Nair and Shyamala Devi, 2006). It also increases the levels of low-density lipoprotein (LDL) cholesterol in the blood, which inturn leads to the build up of harmful deposits in the arteries, thus favouring coronary heart disease (Goldstein and Brown, 1984). Lipid peroxides play an important role in myocardial cell damage. Damage to the myocardium is due to the induction of free radical mediated lipid peroxidation by isoproterenol. Elevated lipid peroxides in isoproterenol induced rats resulted in accumulation of lipids in the heart thereby causing myocardial infarction (Devika and Stanely Mainzen Prince, 2008). Isoproterenol metabolism produces quinones, which react with oxygen to produce reactive oxygen species such as superoxide anions and hydrogen peroxides thereby damaging myocardial cells (Rathore et al., 2000).

In recent years, the prevention of cardiovascular diseases has been associated with the ingestion of fruits, vegetables or plants rich in natural antioxidants. N-Acetyl cysteine ($C_5H_9NO_3S$), a sulfur containing amino acid is an N-acetyl derivative of L-cysteine. It is present in protein rich food and also in the natural food sources such as garlic and onion (Allium plants). N-Acetyl cysteine has potent antioxidant property (Jayalakshmi et al., 2005). Furthermore, N-acetyl cysteine is rapidly absorbed after oral administration in both animals and humans (De Caro et al., 1989). The maximum plasma concentration is reached 2–3 h after administration of N-acetyl cysteine (Bonanomi and Gazzaniga, 1980).

Lipids and lipoproteins play an important role in the pathology of myocardial infarction. To achieve the greatest possible reduction in myocardial infarction risk, treatment strategies should be aimed at reducing the increased levels of circulatory lipids and maintaining the altered levels of lipoproteins (Stanely Mainzen Prince and

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Sathya, 2010). Previously, we reported the protective effects of N-acetyl cysteine on cardiac markers, lactate dehydrogenase isoenzyme pattern, antioxidant system and the *in vitro* free radical scavenging activity in isoproterenol induced myocardial infarcted rats (Nagoor Meeran and Stanely Mainzen Prince, 2011). In continuation of our research on N-acetyl cysteine, in this phase we evaluated the preventive effects of N-acetyl cysteine on lipids and lipoproteins in isoproterenol induced myocardial infarcted rats. The myocardial infarct size was determined by 2, 3, 5-triphenyl tetrazolium chloride test. Also, the *in vitro* total antioxidant effect of N-acetyl cysteine on scavenging 2, 2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{+}$) was studied. In the present manuscript, we investigated more insight into the mechanism of action of N-acetyl cysteine in isoproterenol induced myocardial infarcted rats.

2. Materials and methods

2.1. Drugs and Chemicals

N-Acetyl cysteine was purchased from S.D. Fine Chemicals, Mumbai, India. Isoproterenol hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO, USA. Ferric chloride, activated aluminium oxide, anhydrous ammonium acetate, copper nitrate, isopropanol, perchloric acid, potassium hydroxide, triethanolamine and 2, 3, 5-triphenyl tetrazolium chloride were purchased from Himedia, Mumbai, India. All the other chemicals used were of analytical grade.

2.2. Experimental animals

Male albino Wistar rats (*Rattus norvegicus*) weighing 180–200 g, obtained from the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India were used in this study. They were housed (3rats/cage) in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light and dark cycle at around 22 °C. The rats had free access to tap water and food. They were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The experiment was carried out according to the guidelines of the committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Proposal No: 592; Approval date : 15/12/2008).

2.3. Preparation of myocardial infarcted rats

Isoproterenol (100 mg/kg body weight) dissolved in saline was subcutaneously injected into rats at an interval of 24 h for 2 days (Senthil Kumaran and Stanely Mainzen Prince, 2010). Isoproterenol induced myocardial infarction was confirmed by elevated activities/levels of serum creatine kinase-MB and troponins in rats.

2.4. Experimental design

The rats were randomly divided into four groups of six rats each. Group I: Normal control rats; Group II: Rats were orally treated with N-acetyl cysteine (10 mg/kg body weight) daily for a period of 14 days by an intragastric tube; Group III: Rats were subcutaneously injected with isoproterenol alone (100 mg/kg body weight) at an interval of 24 h for 2 days (on the 15th and 16th days); Group IV: Rats were pretreated with N-acetyl cysteine (10 mg/kg body weight) orally by an intragastric tube daily for a period of 14 days and then subcutaneously injected with isoproterenol (100 mg/kg body weight) for 2 days (on the 15th and 16th days). Normal control and isoproterenol control rats were received saline alone daily for a period of 14 days. N-Acetyl cysteine was dissolved in saline and administered to rats one ml each orally using an intragastric tube daily for a period of 14 days. The dosage (10 mg/kg body weight) and duration of pretreatment (14 days) of N-acetyl cysteine was based on our previous study (Nagoor Meeran and Stanely Mainzen Prince, 2011).

At the end of the experimental period, after 12 h of second isoproterenol injection (i.e. on the 17th day) all the rats were anesthetized with pentobarbital sodium (60 mg/kg body weight) and then sacrificed by cervical decapitation. For serum, blood was collected in dry tubes without anticoagulant. Blood collected in tubes containing heparin was used for the separation of plasma after centrifugation. Heart and liver tissues were excised immediately and rinsed in ice-chilled saline. Heart tissues were also used for histopathology and 2, 3, 5-triphenyl tetrazolium chloride test.

2.5. Assay of serum cardiac marker enzyme and estimation of serum troponins

Activity of serum creatine kinase-MB was measured by a standard commercial kit. The levels of serum troponins T and I were estimated by chemiluminescence immunoassay using standard kits (Roche Diagnostics, Switzerland).

2.6. Estimation/assay of lipids, marker enzyme of lipid metabolism and lipoproteins

Lipids were extracted from the heart tissues by the method of Folch et al. (1957) using chloroform: methanol mixture (2:1 v/v). The levels of total cholesterol, triglycerides and free fatty acids in the serum and heart were estimated by the methods of Zlatkis et al. (1953), Fossati and Prencipe (1982) and Falholt et al. (1973) respectively. Also, the ratio between 3-hydroxy-3 methyl glutaryl-Coenzyme-A and mevalonate in the liver tissue was taken as an index of the activity of HMG-CoA reductase as described by the method of Rao and Ramakrishnan (1975). Plasma thiobarbituric acid reactive substances and lipid hydroperoxides were estimated by the methods of Yagi (1987) and Jiang et al. (1992) respectively. Cholesterol in the lipoprotein fractions was also determined by the method of Zlatkis et al. (1953). High density lipoprotein-cholesterol (HDL-C) was estimated by a standard commercial kit purchased from Agappe Diagnostics, Kerala, India. Low density lipoprotein (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were also calculated as follows:

$$\text{VLDL} - \text{C} = \text{Triglycerides}/5$$

$$\text{LDL} - \text{C} = \text{Total cholesterol} - \text{HDL} - \text{C} + \text{VLDL} - \text{C}$$

2.7. Determination of size of infarcted myocardium

The 2, 3, 5-triphenyl tetrazolium chloride test used for the macroscopic enzyme mapping assay of the infarcted myocardium was done according to the method of Lie et al. (1975). A freshly prepared solution of 1% 2, 3, 5-triphenyl tetrazolium chloride in phosphate buffer was prewarmed at 37-40 °C for 30 minutes in a darkened glass. To remove the excess blood, the heart tissues were washed rapidly in cold water without macerating the tissue. After removing epicardial fat, the left ventricle was taken separately. The heart was transversely cut across the left ventricles to obtain slices not more than 0.1-0.2 mm in thickness. The heart tissue slices were kept in a covered, darkened glass dish containing prewarmed solution of 2, 3, 5-triphenyl tetrazolium chloride and the dish was kept in an incubator and heated to 37-40 °C for 45 minutes. Then the heart slices were turned over thrice and made certain that it remains fully immersed in the 2, 3, 5-triphenyl tetrazolium chloride solution. At the end of the incubation period, the heart slices were kept in fixing solution to fix the tissue. Colour photographs of slices were obtained by a