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Protective effects of sinapic acid on cardiac hypertrophy, dyslipidaemia and altered electrocardiogram in isoproterenol-induced myocardial infarcted rats

Subhro Jyoti Roy, P. Stanely Mainzen Prince*

Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India

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ABSTRACT

Lipids and lipoproteins play a vital role in the pathogenesis of myocardial infarction. There are no studies reported on the protective effects of sinapic acid on changes in electrocardiogram, lipids and lipoproteins in myocardial infarction. This study aims to evaluate the protective effects of sinapic acid on cardiac hypertrophy, dyslipidaemia and alterations in lipoproteins and electrocardiogram in isoproterenol-induced myocardial infarcted rats. Rats were pre- and co-treated with sinapic acid (12 mg/kg body weight) daily for a period of 10 days and were induced myocardial infarction with isoproterenol (100 mg/kg body weight) on 9th and 10th day. Isoproterenol induced rats showed an increased level of serum cardiac troponin-T and elevated ST-segments. Increased levels of serum and heart cholesterol, triglycerides and free fatty acids were observed in isoproterenol induced rats. Isoproterenol also increased serum low density and very low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol. The activity of liver 3-hydroxy-3-methyl glutaryl-coenzyme-A-reductase was elevated in isoproterenol induced rats. The *in vitro* study revealed the potent antioxidant activity of sinapic acid. Pre- and co-treatment with sinapic acid ameliorated cardiac hypertrophy, dyslipidemia and elevated ST-segments in isoproterenol induced myocardial infarcted rats. Thus, sinapic acid prevented the alterations in the levels of lipids and lipoproteins by virtue of its antilipidaemic effect in isoproterenol induced myocardial infarcted rats.

1. Introduction

Myocardial infarction is an important pathological feature resulting in high levels of mortality and morbidity. It, therefore, attracts continuing attention from basic and clinical researchers, epidemiologists and practicing physicians. Myocardial infarction 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 the levels of lipids such as total cholesterol, triglycerides, free fatty acids and phospholipids in the circulation (Nair and Shyamala Devi, 2006). Increased lipolysis and peroxidation of endogenous lipids also play a major role in the cytotoxic action of isoproterenol. It also increases the levels of low density lipoprotein cholesterol in the blood, which in turn leads to the build-up of harmful deposits in the arteries and thus favoring 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.

Many synthetic drugs are used for the treatment of myocardial infarction. However, they cannot meet the demands due to side effects. Thus, a lot of studies have focused on identifying new therapeutic strategies to prevent myocardial infarction. Alternative therapies using phytonutrients are becoming increasingly popular as these preparations have no or minimal side effects and are cost effective. Phenolic acids have received much attention because of their role in the prevention of many human diseases, particularly atherosclerosis and cancer due to their antioxidant properties (Mattila and Kumpulainen, 2002). Sinapic acid, a phenolic acid is a cinnamic acid derivative, which possesses 3,5-dimethoxyl and 4-hydroxyl substitutions in the phenyl group of cinnamic acid. It is widely distributed in the plant kingdom and is obtained from various sources such as rye, fruits and vegetables (Andreasen et al., 2001). Previous scientific studies revealed that sinapic acid exhibits anti-inflammatory (Yun et al., 2008), peroxynitrite scavenging (Zou et al., 2002) and

* Corresponding author. Tel/fax: +914144239141.
E-mail address: ps_mainzenprince@yahoo.co.in (P. Stanely Mainzen Prince).

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neuroprotective effects (Kim et al., 2010). 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. Recently, we reported the dose dependent effects of sinapic acid and its protective effects on lysosomal dysfunction in isoproterenol induced myocardial infarcted rats (Roy and Stanely Mainzen Prince, 2012). In this communication, we reported the protective effects of sinapic acid (12 mg/kg body weight) on cardiac hypertrophy, dyslipidaemia and altered electrocardiogram in isoproterenol induced myocardial infarcted rats.

2. Materials and methods

2.1. Chemicals

Sinapic acid and isoproterenol hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ferric chloride, activated aluminium oxide, copper nitrate, isopropanol, perchloric acid and triethanolamine 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 170–200 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 (three rats/cage) in polypropylene cages ($47 \times 34 \times 20$ 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.

2.3. Preparation of myocardial infarcted rats

Isoproterenol (100 mg/kg body weight) dissolved in 2 mL of saline was subcutaneously injected subcutaneously into rats at an interval of 24 h (Stanely Mainzen Prince, 2011) on 9th and 10th day. The development of myocardial infarction at this dose was confirmed by elevated level of serum cardiac troponin-T (Elecsys troponin-T Stat reagent kit, Product Code. 04660307190, Roche Diagnostics, Mannheim, Germany) in Wistar rats.

2.4. Experimental design

The experiment was performed with four groups of rats, each group consisting of six rats. Group I: Normal untreated rats; Group II: Rats were orally treated with 2 mL of sinapic acid (12 mg/kg body weight) dissolved in 0.5% dimethyl sulfoxide orally using an intragastric tube daily for a period of 10 days; Group III: Rats were injected subcutaneously with isoproterenol (100 mg/kg body weight) dissolved in 2 mL of saline twice at an interval of 24 h (on 9th and 10th day); Group IV: Rats were pre- and co-treated with 2 mL of sinapic acid dissolved in 0.5% dimethyl sulfoxide (12 mg/kg body weight) orally using an intragastric tube daily for a period of 10 days and injected with isoproterenol (100 mg/kg body weight) dissolved in 2 mL of saline twice at an interval of 24 h (on 9th and 10th day). A volume of 2 mL of 0.5% dimethyl sulfoxide was administered alone to normal control (Group I) and isoproterenol control rats (Group III) orally using an intragastric tube daily for 10 days. The dose and duration of pre- and co-treatment of (-) sinapic acid was based on our earlier study (Roy and Stanely Mainzen Prince, 2012).

At the end of the experimental period, after 12 h of second isoproterenol injection (*i.e.*, on 11th day), all the rats were anesthetized with high dose of pentobarbital sodium (60 mg/kg body weight) and then sacrificed by cervical decapitation. Blood was collected in dry test tubes without anticoagulant for serum. Heart and liver tissues were excised immediately and rinsed in ice-chilled saline.

2.5. Estimation of serum cardiac troponin-T

The level of cardiac troponin-T in serum was estimated using a reagent kit by electro chemiluminescence immunoassay (Elecsys troponin-T Stat reagent kit, Product Code. 04660307190, Roche Diagnostics, Mannheim, Germany).

2.6. Electrocardiogram

The electrocardiographic-patterns were recorded by 3 Lead-16 Channel Polygraph (Biopac Systems Inc., USA). The leads were attached to right arm, left arm and left leg of rats. Electrocardiographic recordings were made in anesthetized rats. The types of alterations (ST-segment elevation) in the experimental rats were recorded.

2.7. Estimation/assay of lipids, rate limiting enzyme of cholesterol biosynthesis 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 (HMG-CoA) and mevalonate was taken as an index of the activity of HMG-CoA reductase as described by the method of Rao and Ramakrishnan (1975). 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 (Product No. 11010001, Agappe Diagnostics, Kerala, India). Low density lipoprotein–cholesterol (LDL–C) and very low density lipoprotein–cholesterol (VLDL–C) were also calculated as follows:

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

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

2.8. Statistical analysis

In our study, the data were analyzed by one way analysis of variance followed by Duncan's Multiple Range Test using Statistical Package for the Social Science software package version 12.00. Results were expressed as mean ± standard deviation for six rats in each group. P values < 0.05 were considered significant.

3. Results

Isoproterenol induced myocardial infarcted rats (Group III) showed considerable ($P < 0.05$) increased levels of serum cardiac troponin-T (2.81 ± 0.27 ng/mL) compared to normal control rats (0.18 ± 0.01 ng/mL) (Group I). Pre- and co-treatment with sinapic acid (12 mg/kg body weight) near normalized ($P < 0.05$) the levels