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Preventive effects of *p*-coumaric acid on lysosomal dysfunction and myocardial infarct size in experimentally induced myocardial infarction

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ABSTRACT

The present study was designed to evaluate the preventive effects of *p*-coumaric acid on lysosomal dysfunction and myocardial infarct size in isoproterenol induced myocardial infarcted rats. Male albino Wistar rats were pretreated with *p*-coumaric acid (8 mg/kg body weight) daily for a period of 7 days after which isoproterenol (100 mg/kg body weight) was injected subcutaneously into rats twice at an interval of 24 h (8th and 9th day).The activity/levels of serum cardiac diagnostic markers, heart lysosomal lipid peroxidation products and the activities of lysosomal enzymes (β-glucuronidase, β-galactosidase, cathepsin-B and cathepsin-D) were significantly ($P<0.05$) increased in the serum and heart of isoproterenol induced myocardial infarcted rats. Isoproterenol also lowered the activities of β-glucuronidase and cathepsin-D in the lysosomal fraction. The pretreatment with *p*-coumaric acid significantly ($P<0.05$) prevented the changes in the levels of lysosomal lipid peroxidation products and the activities of lysosomal enzymes. In addition, *p*-coumaric acid greatly reduced myocardial infarct size. *p*-Coumaric acid pretreatment (8 mg/kg body weight) to normal rats did not show any significant effect. Thus, this study showed that *p*-coumaric acid prevents lysosomal dysfunction against cardiac damage induced by isoproterenol and brings back the levels of lipid peroxidation products and activities of lysosomal enzymes to near normal levels. The in vitro study also revealed the free radical scavenging activity of *p*-coumaric acid. Thus, the observed effects are due to *p*-coumaric acid's free radical scavenging and membrane stabilizing properties.

1. Introduction

Myocardial infarction is the acute condition of necrosis of the myocardium as a result of imbalance between coronary blood supply and myocardial demand. Isoproterenol is a synthetic catecholamine, which causes severe stress in the myocardial tissue and its high doses produce acute myocardial necrosis. It has been reported that the free radicals produced by induction of isoproterenol could initiate the membrane bound polyunsaturated fatty acid peroxidation, leading to both functional and structural myocardial injury (Thompson and Hess, 1986). The biochemical mechanism of injury of myocardial cells after myocardial infarction remains unresolved. It is possible to gain more insight into the mechanisms that lead to the altered metabolic process in human myocardial infarction by studying the biochemical alterations in an animal model.

Natural products have a remarkable role in the traditional medicine in different countries. Nowadays prevention of cardiovascular diseases is associated with intake of vegetables and fresh fruits. There is an inverse association between dietary phenolic compounds intake and mortality from various diseases (Sanchez-Moreno et al., 1998). *p*-Coumaric acid (*trans*-4-hydroxycinnamic acid), a phenolic acid, is a hydroxyl derivative of cinnamic acid. It is found in tomatoes, carrots, peanuts, garlic, beans and vinegar. Previous studies have revealed that *p*-coumaric acid exhibits antioxidant property (Halliwell and Ahluwalia, 1976; Castelluccio et al., 1995; Zang et al., 2000). Further, it reduces the risk of stomach cancer (Ferguson et al., 2005).

Lysosomes are cytoplasmic organelles present in animal tissues, which contain hydrolytic enzymes capable of degrading the cellular constituents. They also play a major role in secretion and transport process. It has been reported that the intracellular lysosomal enzymes release and their extralysosomal activity may cause progressive modifications that lead from reversible myocardial ischemia to irreversible infarction (Decker et al., 1977). Also, the lysosomal membrane is a potential site for free radical attack and its leakage can play an initiative role in apoptosis induced by oxidative stress (Ollinger and Brunk., 1995). Cathepsin-D is a lysosomal aspartic protease, which is present in all animal cells. β-Glucuronidase enzyme release is frequently used as an index of lysosomal membrane integrity (Ravichandran et al., 1990).

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Destabilization of lysosomal membrane may be prevented either by cellular peroxidation inhibition or by iron catalyzed oxidative reactions prevention, that involves peroxidation of cellular membranes, depletion of energy and release of lysosomal content (Karthikeyan et al., 2007). The viability of ischemic cardiac muscle may be prolonged by stabilizing the myocardial cell membranes, particularly the lysosomal membranes. Literature survey has shown that there are no scientific studies reported on the effects of *p*-coumaric acid on lysosomal dysfunction in myocardial infarction. Hence, this study was carried out to understand the preventive effects of *p*-coumaric acid on lysosomal dysfunction in myocardial infarcted rats by means of its free radical scavenging and membrane stabilizing properties. The myocardial infarct size was determined by 2,3,5-triphenyl tetrazolium chloride test and quantified by cumulative planimetry. In addition to this, we evaluated *in vitro* effects of *p*-coumaric acid on scavenging superoxide anion and hydroxyl radicals.

2. Materials and methods

2.1. Experimental animals and diet

All the experiments were performed with 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. The rats were housed in polypropylene cages ($47 \times 34 \times 20$ cm) lined with husk, renewed every 24 h under a 12 h light/dark cycle at around 22 °C and were given free access to tap water and food. They were fed on a standard pellet diet (Pranav Agro Industries Ltd., Pune, Maharashtra, India. The diet provided metabolizing energy of 3,600 kcal/kg and the experiment was performed 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 (Approval No. 705; 19th April, 2010).

2.2. Chemicals

p-Coumaric acid, isoproterenol hydrochloride, *N*-phenyl-*p*-phenylenediamine and *p*-nitrophenyl-β-D-glucuronide were purchased from Sigma Chemical Co., St. Louis, MO, USA. Thiobarbituric acid was obtained from S.D. Fine Chemicals, Mumbai, India. 2,3,5-Triphenyl tetrazolium chloride was purchased from Himedia, Mumbai, India. All other chemicals and reagents used in this study were of analytical grade.

2.3. Induction of experimental myocardial infarction and dose dependent study.

Isoproterenol (100 mg/kg body weight) was dissolved in saline and injected subcutaneously into rats twice at an interval of 24 h (Panicker et al., 2010; Kannan and Quine, 2011). Isoproterenol induced myocardial infarction at this dose was confirmed by elevated activity/level of serum creatine kinase-MB and serum cardiac troponin-T.

To determine the dose dependent effect of *p*-coumaric acid in isoproterenol induced myocardial infarcted rats, a pilot study was conducted with three different doses of *p*-coumaric acid (2, 4 and 8 mg/kg body weight).We observed after 7 days of the experiment, *p*-coumaric acid pretreatment at the doses of 2, 4 and 8 mg/kg body weight significantly ($P < 0.05$) lowered the elevated activity of serum creatine kinase-MB in isoproterenol induced myocardial infarcted rats. From this experimental result, it was clear that 8 mg/kg body weight of *p*-coumaric acid exhibited the highest significant ($P < 0.05$) effect and near normalized the activity of creatine kinase-MB with respect to the other two doses (2 and 4 mg/kg body weight). Hence, we have taken 8 mg/kg body weight of *p*-coumaric acid for our further study (Fig. 1).

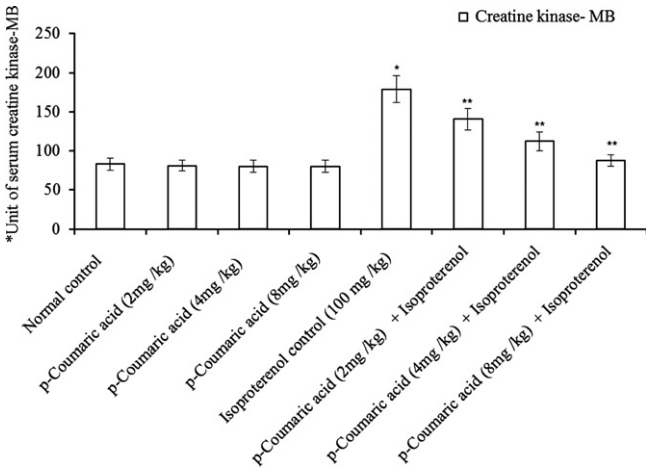

Fig. 1. Activity of serum creatine kinase-MB. Each column is mean ± standard deviation for six rats in each group; *$P < 0.05$ as compared to normal control; **$P < 0.05$ as compared to isoproterenol control (Duncan's Multiple Range Test); *Unit: IU/L.

2.4. Experimental design.

The experimental design consists of four groups of rats, each group consisting of six rats. Group I: Normal control rats. Group II: Rats were treated with 2 mL of *p*-coumaric acid (8 mg/kg body weight) dissolved in 0.5% dimethyl sulfoxide solvent orally by an intragastric tube daily for a period of seven days. Group III: Isoproterenol (100 mg/kg body weight) was dissolved in 2 mL of saline and injected subcutaneously into rats twice at an interval of 24 h to induce myocardial infarction (on 8th and 9th day). Group IV: Rats were pretreated with 2 mL of *p*-coumaric acid (8 mg/kg body weight) dissolved in 0.5% dimethyl sulfoxide orally by an intragastric tube daily for a period of 7 days and then induced with isoproterenol at an interval of 24 h for two days (8th and 9th day). Normal control and isoproterenol control rats were orally given 2 mL of 0.5% dimethyl sulfoxide by an intragastric tube. Twelve hours after the second dose of isoproterenol injection (10th day), all the rats were anesthetized by high dose of pentobarbital sodium (60 mg/kg body weight) and then sacrificed by cervical decapitation. Then, the blood samples were collected from carotid artery in dry test tubes without anticoagulant and allowed to coagulate at ambient temperature for 40 min. Then serum was separated by centrifugation at 2000 rpm for 10 min and stored at −80 °C for the assays of lysosomal enzymes.

2.5. Separation of lysosomal fraction in the heart

The heart tissue samples were cut open and washed in isotonic saline to remove the blood and then rinsed in ice cold 0.25 M sucrose at 4 °C. From this, a portion was taken to assay the total heart lysosomal enzyme activities. Another portion of the heart homogenate was subjected to differential centrifugation to separate lysosomal fraction as follows: structural proteins, nucleus and cell debris at $600 \times g$ for 10 min; mitochondria at $5000 \times g$ for 10 min; lysosomes at $15{,}000 \times g$ for 10 min. Heart lysosomal fractions were treated with Triton X-100 (final concentration 0.2% v/v) in ice for 15 min prior to the assay of various enzyme activities (Sathish et al., 2003).