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Vanillic acid prevents altered ion pumps, ions, inhibits Fas-receptor and caspase mediated apoptosis-signaling pathway and cardiomyocyte death in myocardial infarcted rats

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ABSTRACT

The present study aims to evaluate the preventive effects of vanillic acid on altered ion pumps, ions and Fas-receptor and caspase mediated apoptosis-signaling pathway and cardiomyocyte death in isoproterenol induced myocardial infarcted rats. Male albino Wistar rats were pretreated with vanillic acid (5 mg/kg and 10 mg/kg body weight) daily for 10 days. After the pretreatment, isoproterenol (100 mg/kg body weight) was injected into rats at an interval of 24 h for 2 days to induce myocardial infarction. Isoproterenol induced rats significantly increased activities/levels of serum cardiac markers, plasma lipid peroxidation products, serum uric acid and significantly decreased plasma non-enzymatic antioxidants. Furthermore, isoproterenol significantly altered the activities/levels of ion pumps and ions in the heart. The myocardial expressions of apoptotic genes such as Fas-receptor, caspases-8, -9, and -3 were increased in isoproterenol induced rats. There was a significant increase in cardiomyocyte apoptosis observed in isoproterenol induced rats. Pretreatment with vanillic acid (5 mg/kg and 10 mg/kg body weight) to isoproterenol induced rats showed significant effects on all the biochemical and molecular parameters evaluated. Isolated cardiomyocyte viability by trypan blue exclusion staining also correlated with these biochemical findings. Thus, vanillic acid prevented altered ion pumps, ions and inhibited Fas-receptor and caspase mediated apoptosis-signaling pathway and cardiomyocyte death in isoproterenol induced myocardial infarcted rats. Our study also revealed that pretreatment with vanillic acid at the dose of 10 mg/kg body weight was more effective than 5 mg/kg body weight. The cardioprotective effects of vanillic acid are associated with its antioxidant mechanisms.

1. Introduction

Myocardial infarction is a leading cause of mortality and disability of adults in urban and rural India and occurs at younger age than in western populations [1]. Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Pharmacological induction of myocardial infarction by subcutaneous administration of isoproterenol (ISO) in rats has been found to be convenient because of relatively smaller size of coronary arteries. Isoproterenol hydrochloride, a synthetic catecholamine and β-adrenergic agonist causes severe stress in the myocardium resulting in infarct like necrosis of the heart muscle [2]. ISO induced myocardial necrosis revealed membrane permeability alterations, which cause the functional loss and integrity of myocardial membranes. It also generates excessive free radicals and stimulates lipid peroxidation, which is the causative factor for irreversible damage to the myocardial membrane. The pathophysiological and morphological changes that take place in rat's heart following MI induced by ISO are comparable to the changes taking place in human MI [2].

The ion pumps such as sodium potassium dependent adenosine triphosphatase (Na^+/K^+ATPase), calcium dependent adenosine triphosphatase (Ca^{2+}ATPase) and magnesium dependent adenosine triphosphatase (Mg^{2+}ATPase) play vital roles in the contraction and relaxation cycles of cardiac muscle by maintaining normal levels of Ca^{2+}, Na^+, K^+ and Mg^{2+} within the myocytes. Cellular injury is associated with alterations in ion homeostasis. Changes in the properties of these ion pumps and ions affect cardiac function. Therefore, therapies directed at preventing alterations of these ion pumps and ions in the heart have emerged as attractive avenues of investigation for myocardial infarction [3].

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Cardiomyocyte apoptosis has been well documented in myocardial infarction. There may be a large burst of cardiomyocyte apoptosis that can comprise 5–30% of the total number of cardiomyocytes within the ischemic zone during myocardial infarction. The myocardial apoptosis mediated by reactive oxygen species during reperfusion has been associated with cardiac dysfunction and extension of infarction. Inhibition of this process can delay or even avoid the occurrence of such cardiac abnormalities. Fas – is a transmembrane cell surface receptor that plays a critical role in apoptosis. When engaged by the signaling peptide Fas – ligand, the receptor initiates a cascade that leads to proteolysis and apoptosis [4]. At the center of the apoptosis machinery is a family of intracellular proteases, known as caspases that are directly or indirectly responsible for the morphological and biochemical events that characterize apoptosis. The caspase family consists of more than a dozen caspases (caspase-1 through caspase-14) required for the regulation of apoptosis and inflammation [5]. Functionally, caspases are divided into 3 different types: apoptotic initiators (caspases-2, -8, -9 and -10), executioners (caspases-3, -6 and -7) and cytokine precursors (caspases-1, -4, -5, -11, -12, -13 and -14). Increased expression of apoptosis mediating Fas-receptor and activation of caspases have been suggested as potential mediators of myocyte apoptosis in myocardial infarction [6]. Since apoptosis is implicated in the pathogenesis of myocardial infarction, the inhibition of apoptosis promises to be a potent target for therapeutic intervention.

Phenolic compounds form a substantial part of plant foods. Most of these phenolic compounds are antioxidants *in vi*tro and antioxidants may protect against cardiovascular diseases (CVDs). Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a phenolic derivative of edible plants and fruits and the highest amount is found in the root of *Angelica sinensis*. Studies have shown the antibacterial [7] and antimicrobial [8] properties of vanillic acid. Previously, we reported the preventive effects of vanillic acid on hyperlipidaemia, myocardial infarct size, bax, bcl-2, electrocardiogram, lipid peroxidation and antioxidants in the heart tissue, heart histology and pro-inflammatory markers in ISO induced myocardial infarcted rats [9,10]. In continuation of our research on vanillic acid, in the next phase we evaluated the cardioprotective effects of vanillic acid on serum cardiac markers, lipid peroxides and non- enzymatic antioxidants in the circulation, ion pumps and ions in the heart of ISO induced myocardial infarcted rats. In addition, reverse transcription polymerase chain reaction (RT-PCR) was performed to validate the myocardial expressions of Fas receptor, caspases-8, - 9 and -3 genes involved in Fas-receptor and caspase mediated apoptosis-signaling pathway. Trypan blue exclusion test was performed to know the viability of cardiomyocytes isolated from the rat's heart (Groups I–VI).

2. Materials and methods

2.1. Chemicals and primers

Vanillic acid, isoproterenol hydrochloride, butylated hydroxy toluene and reduced glutathione were purchased from Sigma Chemical Co., St. Louis, MO, USA. Thiobarbituric acid, ferrous ammonium sulfate, sodium pyrophosphate, ethylene diamine tetraacetic acid, phosphotungstic acid, adenosine triphosphate and sodium azide were obtained from Himedia, Mumbai, India. Polymerase chain reaction master mix, Easy Spin column DNA isolation kit, mRNA purification kit, oligo (dt), DNAse and lambda DNA/Hind III were purchased from Medox Biotech India Private Limited, Chennai, India. Primers for reverse transcription polymerase chain reactions were purchased from Ocimum Bio solution Limited, Hyderabad, India. All the other chemicals used in the study were of analytical grade.

2.2. Experimental animals and diet

Male albino Wistar rats (*Rattus norvegicus*) weighing 180–200 g, obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India were used in this study. They were housed (3 rats/cage) in polypropylene cages (47 × 34 × 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. The rats were fed on a standard pelleted 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 (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Proposal No: 699; Approval date: 11-01-2010).

2.3. Induction of experimental myocardial infarction and experimental design

Isoproterenol (100 mg/kg body weight) was dissolved in saline and subcutaneously injected into rats at an interval of 24 h for 2 days to induce myocardial infarction [9,10]. The development of myocardial infarction at this dose was confirmed by elevated activity of serum CK-MB, a diagnostic marker of myocardial infarction in rats. After the second isoproterenol injection, blood was withdrawn by sinocular puncture from rats and collected in tubes without anticoagulant and serum separated. The activity of CK-MB in the serum was assayed by a commercial kit purchased from Agappe Diagnostics, Kerala, India [11]. The rats were randomly divided into 6 groups of 6 rats each. Group I: Normal control rats; Group II: Rats were orally treated with vanillic acid (5 mg/kg body weight) daily for a period of 10 days using an intragastric tube; Group III: Rats were orally treated with vanillic acid (10 mg/kg body weight) daily for a period of 10 days using an intragastric tube; Group IV: Rats were subcutaneously injected with ISO alone (100 mg/kg body weight) at an interval of 24 h for 2 days (on 11th and 12th day); Group V: Rats were pretreated with vanillic acid (5 mg/kg body weight) orally by an intragastric tube daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg body weight) for 2 days (on 11th and 12th day); Group VI: Rats were pretreated with vanillic acid (10 mg/kg body weight) orally by an intragastric tube daily for a period of 10 days and then subcutaneously injected with ISO (100 mg/kg body weight) for 2 days (on 11th and 12th day); Normal control and ISO induced rats were received saline alone daily for a period of 10 days of the experimental period. Vanillic acid was dissolved in saline and administered to rats 1 ml each orally using an intragastric tube daily for a period of 10 days [9,10].

At the end of the experimental period, after 12 h of second ISO injection, (i.e. on 13th day) all the rats were anesthetized and then sacrificed by cervical decapitation. Blood was collected in 2 tubes, one containing ethylene diamine tetra acetic acid (EDTA) for the separation of plasma and another without anticoagulant for the serum. Then, plasma and serum were separated by centrifugation. Heart tissues were excised immediately and rinsed in ice-chilled saline. From the heart, the left ventricle was separated and used for RT-PCR study. Heart tissues from all the groups were also used for the isolation of cardiomyocytes for trypan blue exclusion test.

2.4. Processing of heart tissue

A known weight of the heart tissue was homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (p^H 7.4) solution. The homogenate was