Effect of vanillic acid on ischemia-reperfusion of isolated rat heart: Hemodynamic parameters and infarct size assays

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Vanillic acid is an oxidized form of vanillin produced during the conversion of vanillin to ferulic acid and has free radical scavenging, antioxidant and anti-inflammatory properties. In this study, we investigated the effects of vanillic acid on hemodynamic parameters and infarct size in ischemia-reperfusion of isolated rat heart. Adult male Sprague Dawley rats were randomly divided into control and treatment groups (n=10). The treatment groups were administered vanillic acid 5, 10 and 20 mg/kg orally for 10 days, then the hearts isolated and were exposed to 30 min ischemia and 1 h reperfusion, using langendorff apparatus. The effects of vanillic acid, on left ventricular developed pressure (LVDP), LV end diastolic pressure (LVEDP), LV pressure (LVP), peak rate of rise and fall of LVP (±dp/dt), coronary flow (CF), rate pressure product (RPP) and infarct size were examined. Rats administered with vanillic acid (10 and 20 mg/kg), displayed significantly improved recovery of LVEDP, RPP, LVDP, LVP and ± dp/dt as compared to control group. There was also significant beneficial effect of these two doses to reduce infarct size. Our results suggest that vanillic acid can effectively improve ventricular function and reduce infarct size in ischemia-reperfusion of isolated rat heart.

Keywords: Blood pressure, Coronary flow, Heart attack, Myocardial infarction, Ventricular function.

Clinical procedures such as transplantation, angioplasty and by-pass surgery are the main causes of cardiac ischemia-reperfusion injury. The blood restoration and the introduction of oxygen into the transiently ischemic tissue result in reactive oxygen species (ROS) production and oxidative stress1,2. The measure of oxidative stress and the tissue injury depends on the ability of the cellular antioxidant defense to overcome this oxidative burden. Myocardial injury due to ischemia-reperfusion includes cardiac contractile dysfunction, microvascular damage, arrhythmias, myocardial stunning ‘reversible mechanical dysfunction’ as well as irreversible myocyte damage3. These changes are the outcome of imbalance between the formation of oxidants and the availability of endogenous antioxidants in the heart4. Increasing the formation of reactive oxygen species (ROS) during ischemia–reperfusion and the damaging effects of free radicals on heart tissue have now been well established by both direct and indirect measurements and the role of oxidative stress in ischemia–reperfusion injury and the importance of antioxidant mechanisms in cardioprotection has been established by many4,7.

Vanillic acid, a benzoic acid derivative used as a flavoring agent, is an oxidized form of vanillin produced during the conversion of vanillin to ferulic acid8. Kaur and Chakraborti reported high vanillin yield during biotransformation of ferulic acid to vanillin from rice bran using an isolate of Pediococcus acidilactici9. The highest quantity of vanillic acid in plants has been found in the roots of Angelica sinensis10. Systematic evaluation of vanillic acid, vanillin and ethyl vanillin by multiple assays has demonstrated the superiority of radical-scavenging and antioxidative activity of vanillic acid11. Various studies have shown the effectiveness of vanillic acid in the management of immune or inflammatory responses12, colitis13 and hepatoprotection14. Moreover, vanillic acid has been shown to have significant protective effects on antioxidant system, cardiac troponins, electrocardiogram, expressions of interleukin-1β, interleukin-6, lipid peroxidation and tumor necrosis factor-α gene and also biochemical parameters in the heart of isoproterenol induced cardiotoxic rats15,16. In this study, we investigated possible protective effects of vanillic acid on ventricular dysfunction induced by ischemia-reperfusion of isolated heart by determining
its effects on hemodynamic parameters and infarct size of isolated rat heart.

**Materials and Methods**

**Materials**—Vanillic acid was purchased from Sigma-Aldrich Co. (USA), Ketamine Hcl (10%) and Xylazine (2%) from Alfasan Co. (Holland). Krebs salts were obtained from Merck Co. (Germany).

**Animals and Treatments**—Adult male Sprague Dawley rats (250-300 g body wt.) were randomly divided into 4 experimental groups (n=10 for each group) as follows: group I, control; groups II (V-5), III (V-10) and IV (V-20) which received vanillic acid 5, 10 and 20 mg/kg, respectively. Vanillic acid was suspended in normal saline and was administered to rats via an oral gavage needle for 10 days before experiment. Control group received normal saline orally for the same duration of period. Animals were maintained in the animal house of Jundishapur University of Medical Sciences, Ahvaz, Iran, in accordance with the guidelines and approval of the Animal Care and Use Committee Laboratory Animals of the University (No. ajums.REC.1392.91, Date: 04.05.2013).

**Langendorff heart perfusion experiments**—Rats were anesthetized with i.p. injection of Ketamine (50 mg/kg) and xylazine (5 mg/kg), containing Heparin (1000 U/kg). After cannulation of trachea, rats were ventilated with room air using a rodent ventilator (UGO BASILE, model 7025). The thorax was opened, a silver cannula was inserted into the aorta and tightened with a suture, and the heart was rapidly removed from the body and mounted to a Langendorff perfusion apparatus. The heart was perfused at 37±0.1°C and a constant pressure of 70 mmHg. The perfusion Krebs-Henseleit buffer consisted of 118 mM NaCl, 4.75 mM KCl, 1.75 mM CaCl₂, 1.18 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃ and 11.1 mM glucose in double distilled water, pH 7.4 and equilibrated with 95% O₂+5% CO₂. For each experiment, fresh perfusion buffer was filtered through a 1.2-µm microfibre filter (GF/C glass filters; Whatman). The following cardiac parameters were continuously monitored by a PowerLab system (AD Instruments, Castle Hill, Australia): heart rate (HR), left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), rate pressure product (RPP) that is the product of HRxLVDP, maximal and minimal first derivatives of LVDP as a function of time (±dp/dt), respectively. To assess LVEDP, a water filled latex balloon was placed into the left ventricular cavity through the mitral orifice and connected to a pressure transducer. The balloon was initially inflated to produce an LVEDP of 5-10 mmHg.

After 20-30 min stabilization, necessary to reach the maximal functional cardiac values, hearts were subjected to 30 min of no flow global ischemia followed by 60 min of reperfusion. Indication of ischemia was ST elevation, monitored on ECG.

**Determination of infarct size**—At the end of reperfusion, hearts were frozen and subsequently cut into 2 mm-thick transverse sections. The slices were incubated in a solution contains 1% TTC (tri-phenyltetrazolium chloride) and 0.1 M phosphate buffer (pH= 7.4) at 37°C for 10 min. After incubation in TTC, the slices were fixed in 10% formalin solution for 20 min. Both sides of each slice were then scanned into a computer for planimetric analysis using image analysis software (NIHimagepro.1.16). Because hearts were subjected to global ischemia, the total cross-sectional areas were determined as the total risk areas. The ratio of infarcted area-to-total risk area of the 2 sides of each slice was calculated. The final infarct size of each heart was the average of infarcted area-to-total risk area ratio of all slices.

**Statistical analysis**—The data were analyzed with SPSS version 20 and compared with control values using One way ANOVA and repeated measurement followed by appropriate post hoc according to the experimental protocols. The data were expressed as mean±SEM and were considered significant at a level of \( P < 0.05 \).

**Results**

**Effects of vanillic acid on left ventricular function**

**Left ventricular developed pressure (LVDP)**—The LVDP (LVSP-LVEDP) baseline prior to ischemia in control and treatment groups was not significantly different (Fig. 1A). After ischemia and during reperfusion, all LVDP values declined but for the treated groups III and IV (V-10 and V-20) which exhibited less reduction. Based on the data shown in Fig. 1A, group IV (V-20) demonstrated the best recovery LV contractile function, significantly different as compared to control group (\( P < 0.01 \)) followed by group III (V-10) that showed significant (\( P < 0.05 \)) post-ischemic LVDP recovery compared to the control group.

**Left ventricular pressure**—The LVP baseline prior to ischemia in control and treatment groups was not significantly different. After ischemia and during reperfusion, LVP values of all groups were decreased. However, as shown in Fig. 1B, in V-10 and V-20
groups, the changes in LVP were reduced significantly \( P < 0.05 \) as compared to the control.

**End diastolic pressure**—Comparison of pre-ischemic LVEDP between control and treatment groups (Fig. 1C) showed no significant differences. From the onset of reperfusion, LVEDP of control and group II (V-5) rats rapidly rose and remained high throughout the reperfusion (post-ischemic contracture). The LVEDP of V-10 and V-20 groups (III & IV) not only reduced less than control, but also progressively \( P < 0.05 \) reduced up to the end of reperfusion. Post-ischemic contracture was shown lower in these groups than in control and V-5 groups.

**Rate of the changes in heart contraction and relaxation**—Fig. 2 A and B present the peak rate of rise of LVP \( (+\text{dp/dt}) \) and peak rate of fall of LVP \( (-\text{dp/dt}) \) respectively, before global ischemia and during reperfusion period in the control and treatment groups. These two variables indicate myocardial contractility and relaxation, respectively. There were no significant differences in baseline values of \( \pm \text{dp/dt} \) before ischemia between the control and treatment groups. However, in V-10 and V-20 groups (III & IV), the changes in \( \pm \text{dp/dt} \) were significantly \( P < 0.05 \) attenuated as compared to those of control.

**Effects of vanillic acid on coronary flow**—As shown in Fig. 3A, although during pre-ischemic perfusion, CF was higher in treatment groups than in control, this

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**Fig. 1**—Effect of ischemia (30 min) and reperfusion (60 min) on: (A) LVDP; (B) LVP; and (C) LVEDP in control and treated groups that received vanillic acid (5, 10 and 20 mg/kg) for 10 days \( (n=10) \). Data expressed as Mean ± SEM. Repeated measurement ANOVA was used, followed by LSD test. Level of significance: * \( P < 0.05 \) and ** \( P < 0.01 \).

**Fig. 2**—Effect of ischemia (30 min) and reperfusion (60 min) on: (A) \( +\text{dp/dt} \); and (B) \( -\text{dp/dt} \) in control and treated groups that received vanillic acid (5, 10 and 20 mg/kg) for 10 days \( (n=10) \). Data expressed as Mean ± SEM. Repeated measurement ANOVA was used, followed by LSD test. Level of significance: * \( P < 0.05 \).

**Fig. 3**—Effect of ischemia (30 min) and reperfusion (60 min) on: (A) coronary flow; and (B) RPP in control and treated groups that received vanillic acid (5, 10 and 20 mg/kg) for 10 days \( (n=10) \). Data expressed as Mean ± SEM. Repeated measurement ANOVA was used, followed by LSD test. Level of significance: * \( P < 0.05 \) and ** \( P < 0.01 \).
difference were not statistically significant. Restoration of flow led to rapid recovery of contractile function, but at a lower level than pre-ischemia. Fig. 3A, shows that CF of all groups progressively decreased during the reperfusion period, but groups III and IV (V-10 and V-20) exhibited better recovery of CF that were significantly different as compared to control group ($P<0.05$).

**Effects of vanillic acid on rate pressure product**—RPP is the product of HR×LVDP. As shown in Fig. 3B, there was no significant difference in RPP baseline prior to ischemia in control and treatment groups. The RPP values of all groups gradually lowered during reperfusion. However, V-10 and V-20 groups showed significantly less reduction and better recovery ($P<0.05$ and $P<0.01$, respectively) as compared to control group.

**Effect of vanillic acid on infarct size**—Although we demonstrated the efficiency of vanillic acid in post-ischemic recovery of ventricular function, the question that whether this beneficial and protective effect can protect the myocardium against ischemia–reperfusion tissue injury still remained unanswered. To address this, we compared vanillic acid treatments with control group in infarct size determination experiment. As shown in Fig. 4, vanillic acid reduced infarct size relative to control group. The average infarct size in rat hearts of control group was $28\pm4\%$ of the total risk area, whereas those for the treated groups V-5, V-10 and V-20 it was $26\pm3.5\%$, $21\pm2.8$ and $13\pm3.3\%$, respectively. These results demonstrated that V-10 and V-20 groups had significant differences with control group, ($P<0.05$) and ($P<0.01$), respectively. This finding represents the dose dependent effect of vanillic acid to diminish infarct size.

**Discussion**

In the present study, ischemia–reperfusion (IR) in control group caused systolic and diastolic dysfunction that was associated with impaired contraction and relaxation and also increased infarct size. It has been confirmed that reduction in endogenous antioxidant enzyme activity causes poor functional recovery of cardiac tissue after ischemia–reperfusion$^{18}$. Possibly, this is due to the declined capacity of these tissues to counter the deleterious effects of reactive oxygen species (ROS) generated during reperfusion. Many studies have shown the role of ROS in the pathogenesis of cardiac ischemia–reperfusion$^{19,20}$. ROS generation causes alterations such as depression in contractile function, arrhythmias, change in gene expression, and loss of adrenergic pathways in ischemic-reperfused hearts$^{21}$. Thus, alteration in the myocardium during ischemia–reperfusion is in part due to oxidative stress. Moreover, oxidative stress is closely related to the imbalance between ischemic factors and defensive factors, and also there is an inflammatory response that results in considerable accumulation of polymorphonuclear leukocytes in the myocardium for the first 4-6 h of reperfusion$^{22}$. Hence, we conclude that some pre-treatment to strengthen the defensive factors and to reduce inflammatory response of the heart would be effective to protect myocardium during ischemia-reperfusion. Vanillic acid is a natural substance found in food, drug, beverages and cosmetics and also is an established and potent antioxidant and radical-scavenger$^{11}$. There is also evidence that vanillic acid has anti-inflammatory effect$^{13}$. In this study, we observed valuable changes in the ability of cardiac tissue to recover from ischemia–reperfusion following the administration of 10 and 20 mg/kg vanillic acid. These doses effectively improved systolic and diastolic dysfunction. The significant changes in the LVDP in reperfusion were accompanied by similar significant changes in $\pm dp/dt$ values. Increased LVEDP reflects residual diastolic dysfunction and is representative of the inability of the left ventricle to relax during diastole (as observed in control group). However, significant decrease of LVEDP in V-10 and V-20 groups evidenced improvement of diastolic dysfunction. This was associated with a significant increase in the RPP,
especially in V-20 group. Ischemia-reperfusion exposes the heart to many cell stresses including increased production of ROS, ionic imbalances, metabolic deprivation, and osmotic and mechanical stresses\textsuperscript{23}. Also, many studies have revealed that a burst of oxidant stress takes place during the first few minutes after reperfusion of ischemic myocardium\textsuperscript{24}. Although the origin of these oxidants has not been fully understood, numerous therapeutic strategies that decrease the burst, including therapeutic hypothermia, chemical antioxidants and pre and post-conditioning, are highly protective. These observations strongly mention that the ROS burst at reperfusion is both essential and adequate to cause cell death after ischemia. Myocardium can be adapted against oxidative stress through increase of cellular antioxidant such as catalase, SOD, glutathione and glutathione peroxidase\textsuperscript{25}. As oxidative stress plays a central role in ischemia-reperfusion injury, myocardial protection through adaptation is an effective therapeutic method. Myocardial adaptation takes place in reaction to different kinds of repulsive stimuli, such as ischemia\textsuperscript{26}, reactive oxygen species\textsuperscript{27}, etc. It is obvious that induction of adaptation through these obnoxious stimuli is not an acceptable therapeutic approach. Therefore, pharmacological approaches of myocardial adaptation have recently increased\textsuperscript{28-31}. It is interesting to note that the synthesis of cellular antioxidant is stimulated by different kinds of plant and plant extracts\textsuperscript{32-35}. Previous studies using green tea polyphenol, which was given orally as an antioxidant, have shown that it delivered and remained in the cell membrane of cardiomyocyte and reduced edema in these cells and also improved LV function after ischemia-reperfusion\textsuperscript{34,35}. Similarly, garlic administration has prevented oxidative stress and associated ultrastructural changes induced by myocardial ischemic reperfusion injury\textsuperscript{34}. In accordance with the previous studies, we also showed that vanillic acid helps to maintain good LV function after ischemia-reperfusion and reduce infarct size. Oral uptake is a simple method and hence, it may be useful in clinical treatment. Compared with allopurinol, a chemical antioxidant which inhibits xanthine oxidase\textsuperscript{36}, natural antioxidant, including green tea polyphenol, garlic extract and vanillic acid has some advantages\textsuperscript{34}. These natural substances have no serious side effects while allopurinol has been reported to have some side effects, e.g. headache, drowsiness, etc.\textsuperscript{34}. Hence, vanillic acid can serve as not only a dietary cure method but also a medical treatment for ischemia-reperfusion injury. In this study, we did not find dose dependent effects of vanillic acid on measured variables, except for infarct size.

In conclusion, pre-treatment with vanillic acid, improved significantly LV functional parameters and reduced infarct size in ischemia-reperfusion isolated rat heart by diminishing the oxidative stress that caused ischemia-reperfusion injury.

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