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Vanillylmandelic acid protects against reperfusion injury in an experimental animal model of myocardial infarction

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ABSTRACT

Vanillylmandelic acid, a catecholamine end-metabolite, has been shown to have several biological properties in previous studies, despite considered biologically inactive. We examined the potential effects of vanillylmandelic acid on the ischemic heart following myocardial infarction and reperfusion on a rat model. Thirty-four female Wistar rats were randomized into two groups, control and experimental. They were anesthetized and subjected to myocardial infarction through left anterior descending artery ligation. A previously studied dose of vanillylmandelic acid (10 mg/kg) was administered and the following parameters were studied during ischemia and reperfusion: a) mortality b) severity of ventricular tachyarrhythmias c) premature ventricular contractions and d) heart rate. Administration of vanillymandelic acid significantly reduced the severity of ventricular tachyarrhythmias and mortality rate during reperfusion, while it did not affect any other of the parameters studied. In conclusion, reperfusion injury was blunted through vanillylmandelic acid administration, which seems to be mediated by parasympathetic activation.

1. Introduction

Acute myocardial infarction is a leading cause of morbidity worldwide. For this reason, extensive research is being carried out to understand the mechanisms involved in infarct formation, progress and reperfusion injury, following successful coronary artery recanalization.

The investigation of the role of the autonomous nervous system during myocardial infarction and reperfusion is an area of intense scientific research. Catecholamines are neurotransmitters of the sympathetic nervous system which rise abruptly minutes after the installation of myocardial ischemia [1] and induce the onset of fatal ventricular arrhythmias, such as ventricular tachycardia and ventricular fibrillation as shown in animal models [2]. Augmented sympathetic activity for months after the event in patients who suffered an acute myocardial infarction could partially explain increased mortality, sudden cardiac death and left ventricular dysfunction in these patients [3–5]. Ischemic myocardium is electrically unstable and more sensitive to elevated catecholamine plasma levels and, thus, more susceptible to ventricular arrhythmogenesis mainly through norepinephrine a_1 receptor activation [3,6–12].

The main end-product of catecholamine metabolism is vanillylmandelic acid. Although for a long time it was considered a biologically inactive endogenous substance, certain studies oppose this hypothesis. Vanillylmandelic acid demonstrates free radical scavenging [13] and anticoagulant [14] properties whereas it can act as a vagal stimulator [15]. These features render vanillylmandelic acid a potent candidate for investigating its role on myocardial ischemia.

The aim of the present study was to examine whether vanillylmandelic acid-mediated vagal stimulation and its anticoagulant properties would show cardioprotective potential in an animal model of myocardial infarction.

Abbreviations: CG, control group; EG, experimental group; PVCs, premature ventricular contractions; VT, ventricular tachycardia; VF, ventricular fibrillation.

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2. Materials and methods

2.1. Animal study population

The study was conducted on 34 female Wistar rats (7 ± 1 months of age, weighing 250 ± 20 g). The animals were housed (2 per cage) in our animal facilities, under controlled conditions in terms of humidity (60–70%), room temperature (20 ± 2 °C) and light/dark cycles (12 h day/12 h night); access to standard rat chow (Viozois S.A., Animal Feed Company of Epirus, Greece) and water was unrestricted. The animals received human care and all procedures comply with European legislation (*European Union directive for the protection of animals used for scientific purposes*, 2010/63/EU). The study protocol was approved by the Institutional board and by the responsible state authorities.

2.2. Chemicals

Vanillylmandelic acid was purchased from *Sigma-Aldrich* (St. Louis, MO, USA). Intravenous (26-gauge and 18-gauge) catheters and isoflurane were purchased from *Abbott Pharmaceuticals* (Sligo, Republic of Ireland).

2.3. Anaesthesia, intubation and monitoring

After induction of anaesthesia with diethyl ether, the rats were intubated with an 18-gauge catheter. They were immediately ventilated at a ventilation rate of 90/min and a tidal volume of 4.5 ml. Positive pressure anaesthesia was maintained with a mixture of 21% oxygen (athmospheric air) and 2.5% isoflurane. Continuous 6-lead ECG monitoring (limb leads) was available throughout the procedure.

2.4. Induction of myocardial infarction

Myocardial infarction was induced according to the technique proposed by Pfeffer et al. [16]. Following anaesthesia, a left intercostal thoracotomy was performed and the subcutaneous layers were carefully separated, with the use of atraumatic tools, until the thoracic cavity and the pericardium were exposed. The pericardium was opened with care in order to avoid further heart injury during pericardiotomy. The heart, protruding from the incision, was immobilized with a 6.0 silk suture penetrating the tip of the apex. The left anterior descending artery was ligated with the use of the silk suture. The tips of the suture were passed through a plastic tube, held with a clamp and pressure was applied in order to ensure a complete occlusion of the artery. The heart was, then, placed inside the thoracic cavity for the duration of the occlusion protocol and the ribs were re-approximated. Myocardial infarction was certified by an ST segment elevation, especially in lead I and avL. At the end of the occlusion period the suture was loosened to initiate the reperfusion period.

2.5. Myocardial infarction protocol

The rats were randomly allocated into two groups (n = 17 per group): controls (Control Group, CG) and vanillymandelic acid administration at a dosage of 10 mg/kg (Experimental Group, EG). This dose has been shown to elicit parasympathetic activation in previous experiments in our laboratory [15]. The drug was diluted in 1 ml of Ringer's lactate solution and injected intraperitoneally 10 min before the rats underwent the thoracotomy. The same volume of Ringer's lactate solution was administered at the Control Group.

Throughout the experiment the following parameters were measured:

a) The incidence of premature ventricular contractions (PVCs), namely contractions from ventricular foci, b) The incidence and duration of runs of ventricular tachycardias (VTs) or/and ventricular fibrillation (VF). A run of VT was defined by three or more consecutive ventricular contractions. Venticular fibrillation was defined as a rapid (>300/min), asynchronous, with low amplitude, electrical activity of the ventricular myocardium. Venticular tachycardias, as well as VF, were defined as ventricular tachyarrhythmias, c) heart rate, d) mortality rate.

The 60-minute experiment was separated into two periods: the occlusion period, in which the left anterior descending artery remained occluded, and lasted for 30 min and the reperfusion period (30 min).

2.6. Arrhythmia quantification

Ventricular tachyarrhythmias were quantified using the Curtis scoring system [17]. It is an algorithm, which assigns a number to an arrhythmogenic incident in proportion to its severity; where the higher the number is the more severe the incident. Arrhythmias are assigned to the highest possible value. The score is as follows:

0 = 0–49 PVCS
1 = 5–499 PVCs
2 = more than 499 PVCs and/or 1 episode of spontaneously reverting VT/VF
3 = more than 1 episode of VT/VF less than 60 s total duration
4 = VT/VF, 60–119 sec total duration
5 = VT/VF more than 120 s total duration
6 = fatal VF starting at more than 15 min after occlusion
7 = fatal VF starting at between 4 min and 14 min 59 s after occlusion
8 = fatal VF starting at between 1 min and 3 min 59 s after occlusion
9 = fatal VF starting at before 1 min after occlusion

2.7. Statistical analysis

All values are expressed as mean ± standard error of the mean. The non-parametric data were analysed with the two-tailed Mann-Whitney U test and statistical significance was set at $p < 0.05$. The SPSS version 16.0, Chicago, USA software was used to perform the analysis as well as the GraphPad Prism 6 Software for creating the manuscript figures.

3. Results

3.1. Heart rate

Heart rate was comparable between the two groups (331 ± 4 bpm for CG vs 319 ± 4 for EG) at baseline, at the end of the occlusion period (145 ± 10 bpm vs 184 ± 10 bpm) and reperfusion period (119 ± 11 bpm vs 145 ± 9 bpm).

3.2. Curtis score

The severity of arrhythmias in both groups, as quantified by the Curtis score, is graphically depicted in Fig. 1. Curtis score did not differ during ischemia (2.7 ± 0.10 for CG vs 3.1 ± 0.16 for EG) but differed significantly during reperfusion period (1.4 ± 0.2 vs 0.25 ± 0.10, $p < 0.05$) (Fig. 1)

3.3. Ventricular tachyarrhythmias

No statistical significant difference was recorded between the two groups in the frequency and duration of VTs/VF during the occlusion period. However, a significant difference was observed

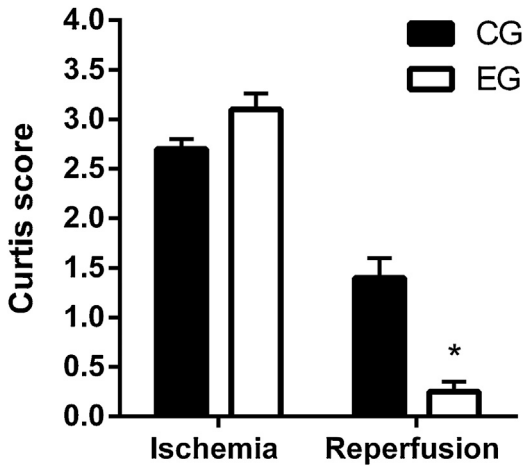

Fig. 1. Curtis score for CG and EG during ischemia and reperfusion periods. CG, Control Group; EG, Experimental Group. * Denotes significant ($p < 0.05$) difference between CG and EG.

in the incidence of ventricular tachyarrhythmias during reperfusion period (Fig. 2). The total duration of the arrhythmias, although similar between the groups during ischemia, differed significantly during reperfusion (21.3 ± 5.3 s for CG vs 0.6 ± 0.1 s for EG, $p < 0.05$) (Fig. 2).

3.4. Premature ventricular contractions

The frequency of PVCs was comparable in the groups studied during, both ischemia (47 ± 6 for CG vs 83 ± 18 for EG) and reperfusion (6 ± 2.3 vs 14 ± 6).

3.5. Mortality rate-mean survival period

During the occlusion period mortality rates were similar in both groups (11.3%, 2 out of 17 animals). However, a significant reduction ($p < 0.05$) was observed during reperfusion in favor of the EG. No remarkable difference was noted in the mean survival period (46 ± 7.1 min for CG vs 51.9 ± 7 min for EG) (Fig. 3).

4. Discussion

In this study, we showed that intraperitoneal administration of vanillylmandelic acid significantly reduced the frequency and severity (as depicted in the Curtis score) of ventricular arrhythmias during reperfusion. In previous experiments we have shown that vanillylmandelic acid abolishes the sympathetic tone when administered at 10 mg/kg, through vagal activation [15]. Pharmaceutically or electrically-induced vagal stimulation seems to display cardioprotective effects in various cardiovascular disorders [18], especially during ischemia and reperfusion [19] strikingly reducing infarction size [20]. Zuanetti et al. studied the effects of vagal stimulation on cat hearts that were subjected to ischemia and showed that vagal activity alleviated ventricular tachyarrhythmias during reperfusion [21]. These effects were also observed in several other animal, as well as clinical studies [22–24]. While catecholamines promote arrhythmias through reentry circuits or increased automaticity [25,26], parasympathetic activation seems to confer protection against this arrhythmogenic substrate, effectively suppressing ventricular tachyarrhythmias, but not PVCs, an effect which was also observed in our study [27,23]

Heart rate was not affected during the ischemic period. Previous experiments in our lab demonstrated that vagal stimulation, and thus significant heart rate reduction, occurs only 40 min following vanillylmandelic acid administration [15]. Considering the fact that the ischemic period only lasted 30 min, one would not expect a measurable heart rate decrease.

Premature ventricular contraction frequency in both groups showed no significant difference. The fact that vanillymandelic acid failed to reduce PVCs remains to be elucidated though, although PVC creation mechanism is multifactorial and catecholamine release is only one of many factors.

Mean survival time was comparable in the groups studied, an obserevation which is linked to the fact that there was no monitoring of survival after 60 min for the animals that had survived until the end of the experiment. According to the protocol, the monitoring ceased after 60 min and the animals had to be euthanized. This explains the lack of mean survival difference, even though there is a significant mortality difference for animals that entered the reperfusion period.

Acute myocardial infarction is a leading cause of mortality and morbidity in the western world. Even with the introduction of primary percutaneous coronary intervention (PPCI) as a therapy, many patients are still at high risk of suffering from reperfusion arrhythmias, especially those that undergo thrombolysis prior to coronary intervention. This is especially dangerous during the transfer of a patient between hospitals after a failed thrombolysis, where reperfusion injury may be a major factor of arrhythmogenesis. Vanillylmandelic acid has shown to reduce such life-threatening arrhythmias and mortality during this period with no apparent adverse effects in the circulatory or other system, as shown by our previous study [15]. This could render vanillylmandelic acid as a candidate for treating reperfusion injury, especially since there is currently no effective therapeutic strategy for this condition [28].

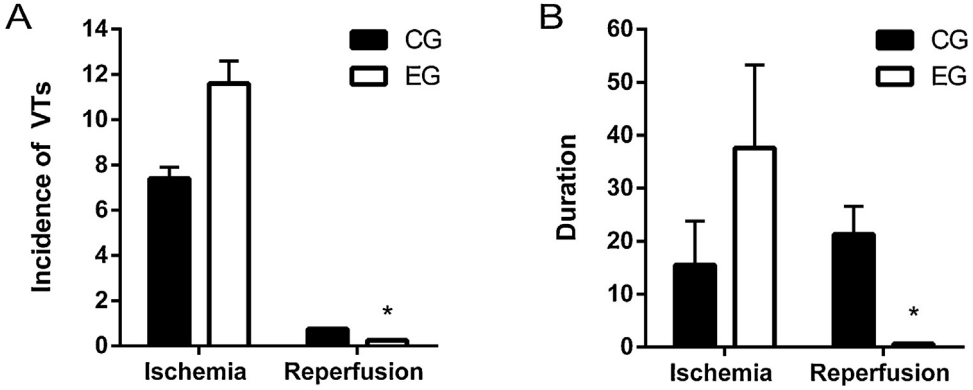

Fig. 2. Ventricular arrhythmias duration for CG and EG during occlusion and reperfusion periods. CG, Control Group; EG, Experimental Group. *, ** Denotes significant ($p < 0.05$) difference between CG and EG for occlusion and reperfusion respectively.

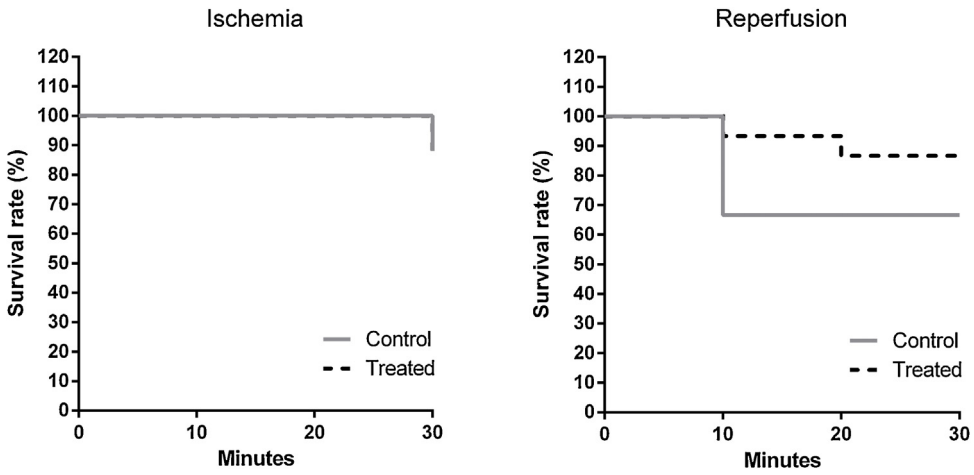

Fig. 3. Survival rate for CG and EG during occlusion and reperfusion periods. CG, Control Group; EG, Experimental Group. * Denotes significant ($p < 0.05$) difference between CG and EG.

However, some limitations should be acknowledged. First, our experiments failed to show a reduction in the overall mortality, although mortality during reperfusion was diminished. Second, this study focused mainly on arrhythmias and mortality and did not quantify the total myocardial injury. This could further illustrate vanillylmandelic acid's cardioprotective properties, because the extent of reperfusion injury directly correlates to myocardial infarct size and prognosis [29,30].

5. Conclusions

In conclusion, vanillylmandelic acid administration prior to myocardial infarction reduced fatal ventricular tachyarrhythmias that ensue during myocardial reperfusion. This effect augments the survival rate only during this period, with no influence on arrhythmogenesis during myocardial ischemia. Further experiments are needed to elucidate this phenomenon.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Declaration of Competing Interest

None.

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