



Research article

Acute administration of nicotine induces transient elevation of blood pressure and increases myocardial infarct size in rats

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ABSTRACT

Aims: We investigated the acute effects of nicotine on myocardial infarct size, no reflow, hemodynamics and cardiac function in an acute myocardial ischemia and reperfusion infarction rat model.**Main methods:** Female Sprague-Dawley rats (n = 23/group) received an intravenous loading dose of nicotine at 2.0 µg/kg/min or saline control for 30 min before starting coronary artery occlusion, then followed by a maintenance dose 0.35 µg/kg/min of nicotine to the end of 30 min occlusion and 3 h reperfusion.**Key findings:** At baseline, there was no difference in systolic blood pressure (BP in mmHg) (nicotine, 69.0 ± 2.7; control, 69.3 ± 4.4; p = NS) or diastolic BP (nicotine, 45.7 ± 3.2; control, 48.2 ± 4.2; p = NS) between groups. Nicotine administration initially increased systolic BP (nicotine, 97.0 ± 8.6; control, 69.2 ± 3.3, p < 0.0001) and diastolic BP (nicotine, 65.6 ± 6.4; control, 47.4 ± 3.1, p = 0.0003) at 10 min after starting injection of the loading dose; BP dropped to control levels in both groups at 30 min. During occlusion and reperfusion, the BP and heart rate were not altered by nicotine. Nicotine significantly increased myocardial infarct size as a percentage of the ischemic risk zone compared to the controls (nicotine, 54.9 ± 1.9; control, 48.6 ± 2.7, p < 0.05), but nicotine did not affect the no-reflow size and heart function.**Significance:** While acute nicotine only transiently elevated blood pressure, it did not affect hemodynamic parameters during coronary artery occlusion. Nicotine increased myocardial infarct size, suggesting that the increase in infarct size was not simply due to an increase in oxygen demand due to altered afterload, heart rate, or contractility, but may have been due to a more direct effect on the myocardium.

1. Introduction

Many studies have demonstrated that cigarette smoking is a risk factor for cardiovascular disease (CVD) [1], particularly for the incident cardiovascular events such as acute myocardial infarction and sudden cardiac death [2]. Nicotine is one component of cigarette smoke that may lead to premature atherosclerosis [3, 4]. Chronic use of nicotine can cause vasoconstriction, stiffen the arterial walls, increase blood pressure, and contribute to reactive oxygen radical damage and endothelial dysfunction [5, 6, 7, 8].

Nicotine is an active component of not only cigarette smoke but is often delivered via electronic cigarette smoking (vaping). Electronic cigarette use has become more common in both young people, who often use it recreationally, and in older people who often use it as a nicotine delivery system to help them quit smoking tobacco [9, 10]. Many of these

older people already have risk factors for cardiovascular disease such as atherosclerosis. Nicotine remains highly addictive, whether delivered by tobacco smoking or by electronic cigarette use [11]. Although it has been suggested nicotine contributed to atherosclerosis and myocardial infarction, few studies have examined the effect of nicotine on the pathology and physiology regarding acute myocardial infarction [12, 13, 14, 15, 16, 17]. The effect of nicotine or tobacco on the size of myocardial infarction reported in the literature has been variable [12, 13, 14, 15, 16, 17]. There is a general lack of knowledge of what effect nicotine has on infarct size, no reflow, hemodynamics and cardiac function in the setting of an acute myocardial infarction. The purpose of the present study was to deliver a clinically relevant dose of nicotine in a standardized model of acute myocardial infarction in rats and determine the effect of nicotine on myocardial infarct size, no reflow, hemodynamics and cardiac function.

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2. Methods

2.1. Animals

The animal protocol was approved by the Institutional Animal Care and Use Committees at Huntington Medical Research Institutes. All experimental procedures carried out in this study were performed in accordance with the guidelines for the care and use of laboratory animals (NIH publication No. 85-23, National Academy Press, Washington DC, revised 2011).

2.2. Animal experimental groups and nicotine administration

In this study, female Sprague-Dawley (SD) rats were used for the experiment as the primary endpoint was myocardial infarct size. We used females because there was no gender difference on the extent of myocardial injury in the setting of experimental myocardial infarction in rats in our previous studies [18]; but we observed that lethal reperfusion-induced arrhythmias led to a higher mortality in male rats than female rats [19]. Forty-six female SD rats (body weight at 225–250 g) were randomly divided into nicotine group and control group ($n = 23/\text{group}$). A clinically relevant dose of nicotine was used as described in a study by Mayhan et al [20]. The rats in the nicotine group were first given a loading dose of nicotine at 2.0 $\mu\text{g}/\text{kg}/\text{min}$ by left jugular vein infusion for 30 min, then followed by a maintenance dose of nicotine at 0.35 $\mu\text{g}/\text{kg}/\text{min}$ for 3.5 h until the end of the experiments. The control group received the same volume of fluid as saline without nicotine.

2.3. Rat model of acute myocardial ischemia and reperfusion

A rat model of left coronary artery occlusion and reperfusion was used as previously described [21]. Briefly, the experimental rats were anesthetized intraperitoneally with ketamine (90 mg/kg) and xylazine (10 mg/kg), and they were intubated and ventilated on a Harvard Rodent Respirator (model VentLite). The rats were kept on a heating pad to maintain body temperature at $\sim 37^\circ\text{C}$. Body temperature and electrocardiogram (ECG) were monitored throughout the entire experimental procedure. After the right carotid artery and left jugular vein were exposed, a pressure sensor catheter (Millar SPR-869-840-8136) was placed into the right carotid artery for blood pressure monitoring and a catheter was placed into the left jugular vein for delivery of nicotine and injection of dyes.

A thoracotomy was performed in the left fourth intercostal space to expose the anterior surface of the heart. After the pericardium was removed, the proximal left coronary artery was encircled with a 4-0 silk suture that was threaded through a small plastic tube. The occlusive site of the coronary artery was located in the interventricular groove just under the tip of the left atrial appendage. The coronary artery was physically occluded by pulling the suture through the plastic tube and clamping the end of the tube. The occlusion was confirmed by the presence of cyanosis on the surface of the anterior-apical portion of the left ventricle and change in the electrocardiogram. Reperfusion was induced by release of the suture.

2.4. Animal experimental procedure

The rats were first allowed to stabilize for approximately 10 min before the experiment started. Then the rats were randomly assigned to either nicotine or vehicle group. A loading dose of nicotine was given by left jugular vein for 30 min before starting coronary artery occlusion, then followed by a maintenance dose of nicotine to the end of 3.5 h of occlusion (30 min) and reperfusion (3 h). 2D and M mode echocardiogram were recorded as described below. Body temperature, ECG and blood pressure were also monitored during the duration of the experimental procedure.

2.5. Evaluation of myocardial ischemic risk area, no-reflow area, and infarct area

During the last few minutes of reperfusion, a fluorescent dye, thioflavin S solution, was injected into the jugular vein to assess the distribution of the no-reflow area. Then a blue dye (Super Imperse Blue) was injected into the jugular vein with the coronary artery re-occluded in order to delineate the ischemic risk area. Blue dye circulated only to the perfused areas and did not reach the ischemic zone. Thus, the non-blue stained area represents the anatomic ischemic risk zone, while the blue stained area represents the non-ischemic zone. At the end of this step, intravenous potassium chloride (KCl) was administered in order to stop the heart in a relative diastolic state, while the rats were under deep anesthesia. After the heart was excised, it was transected into 4 transverse slices from apex to base. The heart slices were photographed under white light to distinguish the ischemic risk zone (pink) in contrast to the nonischemic regions (blue) that received the blue dye. The heart slices were photographed under UV light to distinguish the areas of perfusion by Thioflavin S (fluorescent areas) versus the no-reflow zones (non-fluorescent). Finally, the hearts were incubated with 1% triphenyl tetrazolium chloride (TTC), a chemical that stains viable cells brick red, while dead or necrotic cells appear white to yellow. The photographs were used for planimetry to determine the percentage of each heart slice that was at risk, infarcted, or contained no-reflow. Planimetric areas were corrected by the weight of each heart slice, and then the percentage of each left ventricle that was at risk (ischemic), demonstrated no-reflow and necrosis (infarcted) was calculated. Infarct size and no-reflow zone were expressed as the percentage of the left ventricular ischemic risk zone mass.

2.6. Measurements of hemodynamic and heart function

Hemodynamic data including systolic blood pressure, diastolic blood pressure, mean blood pressure, and heart rate were analyzed at baseline (before nicotine infusion), at 10min and 20min after nicotine infusion, before occlusion, end of occlusion and end of reperfusion. Echocardiography (15-MHZ transducer and Sonos 5500 ultrasound system, Philips Medical System, Andover, MA) was obtained for measurements of end-diastolic diameters and end-systolic diameters at the midpapillary muscle level, LV wall thickness and LV fractional shortening were measured. Echocardiographic data were analyzed from the average of three consecutive beats at baseline, before occlusion, end of occlusion and end of reperfusion.

2.7. Statistical analysis

All data are presented as mean \pm SEM. Mixed effects general linear models were used to examine group differences in changes in outcome measures (systolic pressure, diastolic pressure, mean blood pressure, and heart rate as well as cardiac function parameters), including treatment group as the between-subject factor, time as the within-subject factor, and the interaction term between time and treatment group. Follow-up analyses examined differences between the two groups at specific time-points, as well as within-group changes. The values of myocardial ischemic risk zone, no-reflow area, and infarct size between the two groups were compared with Student's t-test. Statistically significant differences were established at $p < 0.05$.

3. Results

3.1. Effect of nicotine on blood pressure and heart rate

First, blood pressure (BP) and heart rate were analyzed by mixed effects general linear models. Follow-up analyses examined differences between the two groups at specific time-points, as well as within-group changes at baseline before nicotine infusion, after nicotine infusion 10

min (mins) and 20 min, before occlusion, end of occlusion and end of reperfusion. Figure 1 shows the alteration of BP and heart rate at different time points. At baseline, there were no differences between the two groups in mean BP (nicotine, 55.9 ± 3.2 ; control, 57.3 ± 4.4 ; $p = \text{NS}$, Figure 1A), systolic BP (nicotine, 69.0 ± 2.7 ; control, 69.3 ± 4.4 ; $p = \text{NS}$, Figure 1B) and diastolic BP (nicotine, 45.7 ± 3.2 ; control, 48.2 ± 4.2 ; $p = \text{NS}$, Figure 1C) However, after the first 10 min of loading dose of intravenous nicotine, mean BP (nicotine, 78.5 ± 7.2 ; control, 56.7 ± 3.3 ; $p < 0.0001$, Figure 1A), systolic (nicotine, 97.0 ± 8.6 ; control, 69.2 ± 3.3 ; $p < 0.0001$, Figure 1B) and diastolic (nicotine, 65.6 ± 6.4 ; control, 47.4 ± 3.1 , $p = 0.0003$, Figure 1C), were increased compared to controls. The absolute mean BP was increased 22.9 ± 3.0 mmHg at 10 min. This elevated BP then dropped at about 20–30 min and then showed no significant difference between the two groups ($p = \text{NS}$, Figure 1A,B,C). During the infusion of the maintenance dose of nicotine, there were no difference between two groups in mean BP, systolic BP and diastolic BP measured at the end of occlusion (one-hour time point of the experiment, $p = \text{NS}$, Figure 1A,B,C) and at the end of reperfusion (four-hour time point of the experiment, $p = \text{NS}$, Figure 1A,B,C). In addition, we found that nicotine did not alter the heart rate between two groups ($p = \text{NS}$, Figure 1D); but in the nicotine group, there was a significant increase in heart rate from baseline (198.8 ± 9.4) to infusion at 10 mins (218.7 ± 7.3 , $p = 0.003$), and infusion at 20 mins (221.2 ± 10.4 , $p = 0.01$).

3.2. Effects of nicotine on anatomic risk zone, infarct size and no reflow area

The primary aim of this study was to understand whether nicotine can acutely impact the myocardial infarct size and the size of the no reflow zone in an acute myocardial ischemia and reperfusion rat model. We found that nicotine did not alter risk area (nicotine, 52.1 ± 1.6 ; control, 54.0 ± 1.4 , data presented as a percentage of the mass of the left ventricle, $p = \text{NS}$, Figures 2 and 3); but significantly increased infarct size (data presented as a percentage of the necrosis mass of the ischemic risk zone) compared to controls (nicotine, 54.9 ± 1.9 ; controls, 48.6 ± 2.7 , $p < 0.05$, Figures 2 and 3). The size of the no reflow zone (data presented as

a percentage of the mass of the ischemic risk zone) was not affected by nicotine (nicotine, 34.8 ± 3.1 ; control, 33.7 ± 3.0 , $p = \text{NS}$, Figures 2 and 3).

3.3. Effect of nicotine on cardiac function evaluated by echocardiography

The cardiac function was evaluated from echocardiographic measurements of left ventricular internal systolic and diastolic diameter, left ventricular fraction shortening (LVFS), systolic and diastolic left ventricular wall thickness. We found that there was no difference between the two groups for these measurements at baseline, before occlusion, the end of occlusion and the end of reperfusion ($p = \text{NS}$, Table 1). Within group, LVFS in both control and nicotine showed significant change from baseline to the end of reperfusion, from pre-occlusion to the end of reperfusion, and from the end of occlusion to the end of reperfusion ($p < 0.05$, Table 1).

4. Discussion

In this study, an acute intravenous loading dose of nicotine transiently elevated blood pressure at 10 min; with resolution to control levels by 30 min. Overall, nicotine did not alter the heart rate and parameters of cardiac function just before or during experimental acute myocardial ischemia and reperfusion. However, nicotine significantly increased myocardial infarct size in the acute myocardial ischemia and reperfusion rat model, compared to controls. Nicotine did not increase the no reflow phenomenon. The fact that nicotine increased infarct size at a time that the blood pressure and heart rate were not elevated, and without altering cardiac function, suggests that this phenomenon was not simply due to an increase in oxygen demand due to changes in blood pressure or heart rate or contractility. The results suggest that nicotine may be having a more direct negative effect on the myocardium itself.

Nicotine is a major bioactive component in tobacco cigarettes and electronic cigarettes. Plasma nicotine level can be significantly elevated after using 5 min or the fourth 10-puff bouts in electronic cigarette users [22, 23]. Studies have demonstrated that the hemodynamic effects of

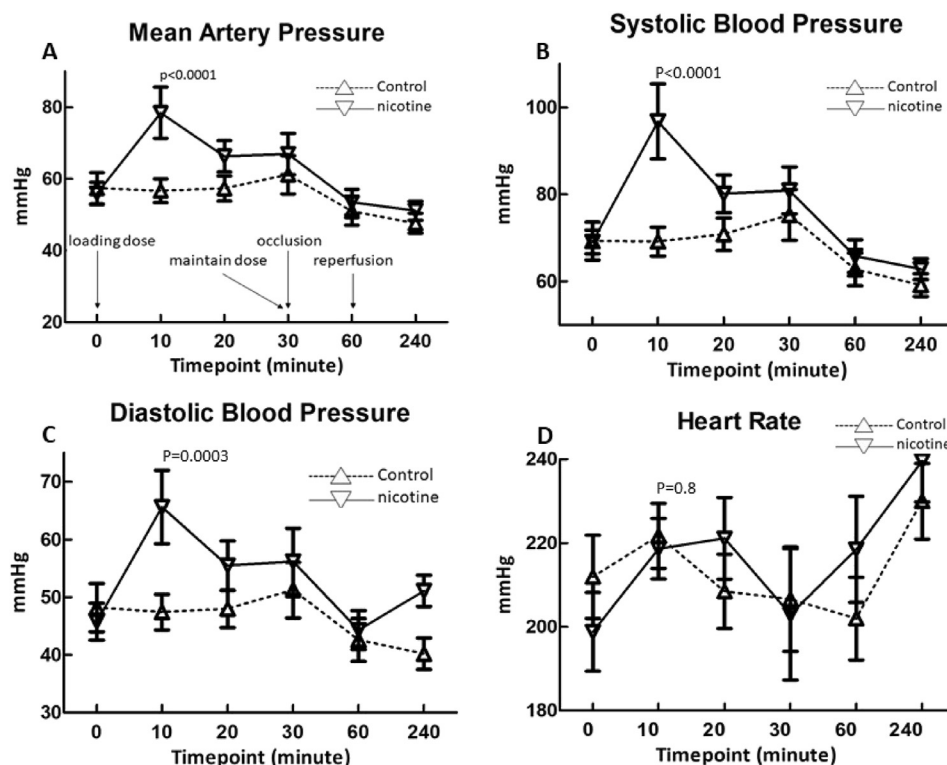


Figure 1. Alteration of hemodynamics. Blood pressure and heart rate were measured after rats ($n = 23$ /each group) were allowed to stabilize for approximately 10 min before the experiment started. A: The time course of mean arterial pressure was significantly different for the 2 groups (group \times time interaction term, $F(5, 215) = 4.56$, $p = .0006$, $t(215) = 4.11$, $p < 0.0001$ at infusion 10 min timepoint); B: The time course of systolic arterial pressure was significantly different for the 2 groups (group \times time interaction term, $F(5, 215) = 5.0$, $p = 0.0002$, $t(215) = 4.89$, $p < 0.0001$ at infusion 10 min); C: The time course of diastolic arterial pressure was significantly different for the 2 groups (group \times time interaction term, $F(5, 215) = 4.54$, $p = 0.0006$, $t(215) = 3.68$, $p = 0.0003$ at infusion 10 min); D: The time course of heart rate was not significantly different for the 2 groups (group \times time interaction term, $F(5, 215) = 2.20$, $p = 0.06$). Further, the control group showed a smaller increase from baseline to post-reperfusion (estimate of the difference = $18.8(11.2)$, $t(215) = 1.68$, $p = 0.1$); in the nicotine group, there was a significant increase in heart rate from baseline to Infusion 10 mins (difference = $19.8(6.6)$, $t(215) = 3.02$, $p = 0.003$), Infusion 20 mins (difference = $22.3(8.6)$, $t(215) = 2.61$, $p = 0.01$).

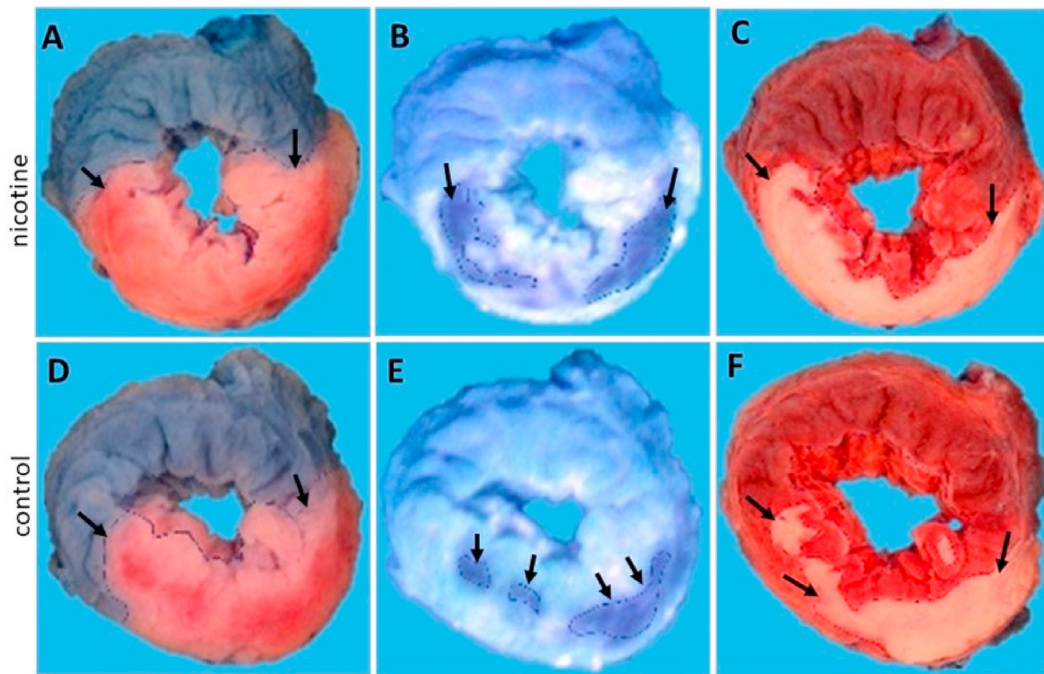


Figure 2. Representative left ventricular slices showing the risk zone, infarct zone, and zone of no-reflow of the heart. Heart slices in panels A and D show the risk area. Blue represents the region that received blue dye during occlusion and is therefore the nonischemic area. The pink area did not receive blue dye and represents the ischemic risk area (arrows). Panels B and E show the no-reflow area (non-fluorescent). The heart slice shown under UV light to distinguish areas of no reflow area (non-fluorescent) from the areas receiving flow during reperfusion (fluorescent). Non fluorescent perfusion defect that did not receive thioflavin S is visualized as dark blue zone (arrows). Panels C and F show infarct area. Heart slice after incubation in TTC to visualize viable tissue (brick red) versus necrosis or infarct tissue (white to light pink). The infarct areas appear as a homogeneous white area (arrows) and is larger in the nicotine treatment heart slice (C) compared to the control (F). The upper row is the nicotine treated heart; The lower row is the control heart.

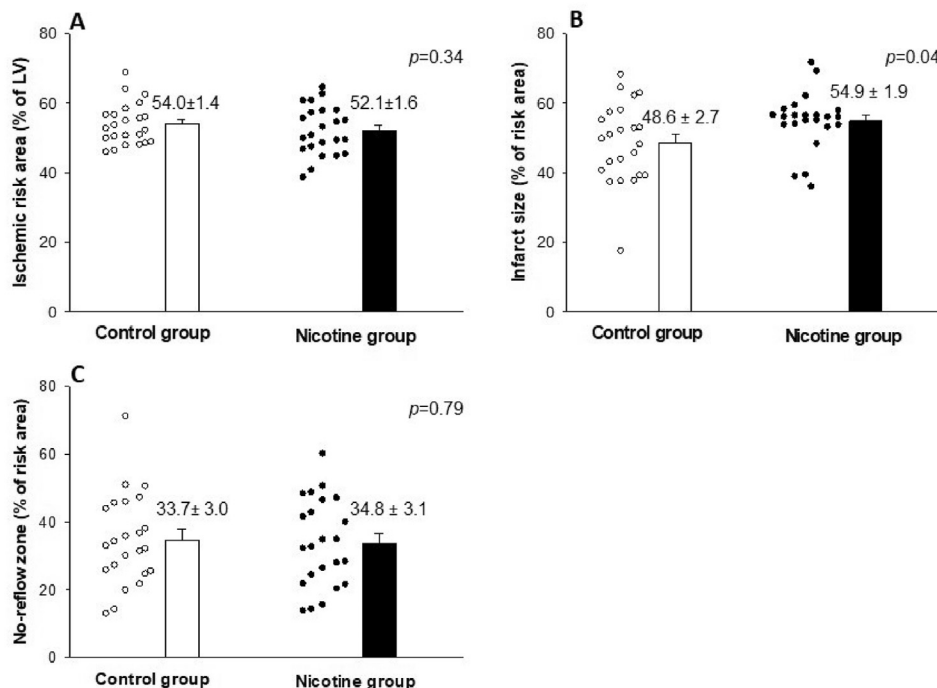


Figure 3. Nicotine increased myocardial infarct size. Rats were randomized into control and nicotine group (n = 23/each group). Risk area, expressed as a percentage of the mass of the left ventricle, was not significantly different between two groups (A). Infarct size, expressed as percentage of ischemic risk area, was significantly increased in nicotine group compared with control group (B). The size of the no reflow zone, expressed as percentage of ischemic risk area, was not significantly increased in nicotine group compared with control group (C).

electronic cigarette use are consistent with what is expected from the effects of nicotine [24]. Any variability of the effects across studies is likely to be related to differences in device-specific delivery of nicotine [11, 25]. Thus, it is important to consider the effect of nicotine when evaluating the potential cardiovascular effects of electronic cigarette use. In humans, nicotine delivered by either tobacco cigarette smoke or

electronic cigarette vapor, can acutely increase blood pressure (BP) [26]. Elevated BP is one of the best established risk factors contributing to clinical cardiovascular disease events such as acute myocardial infarction and mortality [27]. It has been observed that cigarette smoking can transiently increase BP within 5–10 min, but then this effect can decline and stabilize, even while the nicotine levels continue to increase [28]. In

Table 1. Cardiac function.

Baseline	Control	Nicotine	P-value
	N = 23	N = 23	
Systolic ID (mm)	3.03 ± 0.17	2.92 ± 0.20	0.68
Diastolic ID (mm)	5.59 ± 0.23	5.46 ± 0.22	0.70
LVFS (%)	46.30 ± 1.66*	47.52 ± 2.05 ^{&}	0.65
Systolic LV wall thickness (mm)	3.48 ± 0.09	3.56 ± 0.09	0.52
Diastolic LV wall thickness (mm)	2.31 ± 0.08	2.50 ± 0.08	0.08
Before occlusion			
Systolic ID (mm)	2.26 ± 0.14	2.51 ± 0.16	0.24
Diastolic ID (mm)	4.52 ± 0.16	4.76 ± 0.18	0.31
LVFS (%)	50.60 ± 2.27 [#]	48.52 ± 2.35 ^{&}	0.53
Systolic LV wall thickness (mm)	3.73 ± 0.10	3.76 ± 0.10	0.85
Diastolic LV wall thickness (mm)	2.68 ± 0.09	2.67 ± 0.11	0.92
End of occlusion			
Systolic ID (mm)	2.55 ± 0.16	2.59 ± 0.12	0.86
Diastolic ID (mm)	5.01 ± 0.20	5.13 ± 0.16	0.65
LVFS (%)	50.14 ± 2.02 [§]	49.53 ± 1.71 [‡]	0.82
Systolic LV wall thickness (mm)	3.87 ± 0.10	3.81 ± 0.11	0.71
Diastolic LV wall thickness (mm)	2.65 ± 0.09	2.62 ± 0.11	0.81
End of reperfusion			
Systolic ID (mm)	3.23 ± 0.22	3.08 ± 0.21	0.59
Diastolic ID (mm)	5.15 ± 0.20	5.05 ± 0.23	0.77
LVFS (%)	38.54 ± 2.53	40.07 ± 3.37	0.72
Systolic LV wall thickness (mm)	3.63 ± 0.10	3.70 ± 0.16	0.71
Diastolic LV wall thickness (mm)	2.58 ± 0.09	2.64 ± 0.14	0.69

Note: *baseline vs. end of reperfusion, $p = 0.010$; [#] before occlusion vs. end of reperfusion, $p = 0.001$; [§] end of occlusion vs. end of reperfusion $p = 0.002$. [&] baseline vs. end of reperfusion, $p = 0.013$; [‡] before occlusion vs. end of reperfusion, $p = 0.020$; [‡] end of occlusion vs. end of reperfusion $p = 0.008$. LVFS: left ventricular fraction shortening; LV: left ventricle.

our current animal study, nicotine acutely increased BP after 10 min administration followed by a rapid return of BP to control levels, suggesting a pattern that may resemble human acute response to tobacco cigarettes and electronic cigarettes with nicotine [28, 29]. In addition, many studies have shown the variable changes of heart rate with nicotine, including tachycardia [30, 31], bradycardia [32, 33] or no significant changes [17, 34]. Our experimental results did not show significant changes in heart rate compared to control, which might relate to different dosing of nicotine, the fact that our rats were anesthetized, or other factors.

Acute myocardial infarction is the most common type of acute myocardial injury in cardiovascular diseases, in which myocardial ischemia and reperfusion phenomenon occurs, and some groups postulate that reperfusion per se can induce cardiomyocyte death [35, 36]. In this study, we observed that, a low maintenance dose of nicotine given during the acute myocardial ischemia and reperfusion period, increased myocardial infarct size. This suggested that nicotine might enhance the pathological process of the acute myocardial ischemia and reperfusion. Here, the increase in infarct size seems unlikely secondary to a simple increase in oxygen demand, since at the time of coronary artery occlusion and reperfusion, blood pressure in the nicotine group had fallen back toward control levels. Thus, it is possible that nicotine might have a direct negative effect on the myocardial cells that were injured by ischemia/reperfusion. One of the speculated molecular mechanisms might involve an alteration of acetylcholine and/or acetylcholine receptors [37]. The homomeric $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) are expressed in cardiomyocytes, endothelial and inflammatory cells [38]. In humans, activation of $\alpha 7$ nAChRs has been shown to promote endothelial dysfunction, inflammation, and changes in the myocardium [38, 39, 40, 41]. Although we did not specifically study this mechanism in our current protocol, many studies have demonstrated that nicotine can directly impact cardiac structure and function, promoting inflammation, oxidative stress, cardiomyocyte apoptosis and endothelial

dysfunction [42, 43, 44, 45, 46, 47, 48, 49, 50, 51]. In addition, in a recent study by Ramalingam et al, nicotine chronically given for 28 days induced significant increases in blood pressure, heart rate change, cardiac hypertrophy, fibrosis, inflammation, and oxidative stress in myocardial ischemia and reperfusion injury in a rat model, and these effects were partially attenuated by concomitant treatment with irbesartan, an angiotensin II receptor antagonist [52].

A few studies have investigated the effect of nicotine or tobacco smoking on acute myocardial infarct (AMI) size in animal models. Among these studies, several, but not all, have shown that exposure of nicotine or tobacco smoking can increase infarct size [12, 13, 14, 15]. In a study by Sridharan MR et al study, one group of mongrel dogs was exposed to 800 $\mu\text{g}/\text{min}$ of nicotine for 15 min for 14 days and a control group was unexposed before AMI was produced. Nicotine significantly increased infarct size. Dogs exposed to an additional acute dose of nicotine 15 min after onset of AMI or that were exposed daily for an additional week after induction of AMI had even larger infarcts [12]. This study demonstrated that acute, chronic, and post-AMI exposure to nicotine have an adverse effect on infarct size and continued exposure after AMI further enlarged the infarct size. Zhu B. et al reported two in vivo studies, in which SD rats were exposed to tobacco smoking for 3 days, 3 weeks, or 6 weeks (four Marlboro cigarettes/per 15 min, 6 hours/per day, 5 days/per week). Tobacco smoking increased infarct size in a dose dependent fashion and infarct size nearly doubled with 6 weeks of tobacco smoke exposure [13]. Passive exposure to secondhand smoke for 6 weeks, increased infarct size by 35.9% [14]. Similarly, in a study by Ashwani K Khanna, et al, SD rats were exposed to tobacco smoke for 6 weeks (4 cigarettes/day); infarct size increased 21.5%, and LV function deteriorated [15]. Interestingly, in Sofia-Iris Bibli, et al study, male mice were exposed to tobacco smoke (5 cigarettes/time, 4 times/day) for 4 weeks. Compared to other studies this regimen did not increase infarct size, but did abrogate the beneficial effect of ischemic postconditioning [16]. In our previous myocardial ischemia and reperfusion injury rabbit model, acute administration of

intravenous nicotine around 10 min before coronary artery occlusion, but not followed by a maintenance dose of nicotine during myocardial ischemia/reperfusion period, did not affect myocardial infarct size [17]. The differences between these findings compared to our current study in rats may be related to different doses of nicotine, differences in the timing of administration of nicotine (preconditioning) and different animal species.

In the present study, we observed that chronic exposure to nicotine was not necessary to observe and increase in myocardial infarct size. Acute exposure at the time of ischemia was sufficient to increase the size of myocardial infarction and this finding has important clinical implications, especially for those who use vaping as a delivery mechanism of nicotine.

Our experimental results showed that there was a significant increase (by 13%) in infarct size with nicotine exposure while there were no changes in heart function. This suggested that a loading-dose following a low-dose infusion of nicotine did not exacerbate the dysfunction of myocardium initially 'stunned' by the ischemic-reperfusion injury at 3.5 h. Although we did not study long-term recovery of function, it is possible that had we tracked recovery of function longer term, stunned myocardium may have recovered function in the control group. Since more myocardium was necrotic in the nicotine group we may have observed less recovery of function out at weeks to months in that group [53].

In humans, many studies have demonstrated that tobacco smoking can markedly enhance the risk of cardiovascular diseases, including myocardial infarction and sudden death [2, 54, 55]. Meanwhile, there is increasing clinical evidence that electronic cigarettes may be associated with adverse cardiac events. A major component of many electronic cigarettes includes the addition of nicotine. Nicotine is used by both young smokers who are using electronic cigarettes recreationally as well as older smokers who may be using nicotine delivery through electronic cigarettes as a way to quit cigarette smoking. The amount of nicotine that is delivered by electronic cigarette devices is often large; and a single electronic cigarette device may deliver the amount of nicotine that is equivalent to smoking an entire pack of cigarettes. Our study has important clinical implications and raises the concern that vaping that includes nicotine could induce increased myocardial cell necrosis in the setting of infarction. The older person who is vaping with nicotine as a way to quit smoking may especially be at risk as he/she already has the cardiovascular risk factors of being older and having a history of tobacco smoking, both of these are risk factors for developing myocardial infarction. If they vape temporally related to an episode of myocardial ischemia then the tobacco exposure could worsen their situation by increasing myocardial cell death, thus putting them at higher risk of heart failure or even death.

In addition, there is mounting clinical evidences showing that acute electronic cigarette use is closely associated with increased blood pressure, arterial stiffness, oxidative stress, endothelial dysfunction, and vascular injury. Increasing data supports the concept that electronic cigarettes are becoming a serious cause of clinical cardiovascular disease [2, 51, 56, 57, 58, 59, 60]. Many clinical studies indicate that most of the cardiovascular effects of tobacco smoke or electronic cigarette smoke are consistent with the known effects of nicotine.

A recent large study of the long-term cardiovascular implications of using electronic cigarettes from The National Health Interview Surveys of 2014 (n = 36,697) and 2016 (n = 33,028) investigated the cross-sectional association between electronic cigarette use and myocardial infarction, which found daily electronic cigarette use was independently associated with increased odds of having had a myocardial infarction (OR = 1.79). This demonstrated that daily electronic cigarette users were 1.79 times more likely to experience myocardial infarction than never users [61]. Another recent systematic review and meta-analysis of data from January 2000 to November 2017 assessed the long-term cardiovascular effects of the electronic cigarette. Despite some limitations of this analysis, the results suggested that the electronic cigarettes negatively affected endothelial function, arterial stiffness and the long-term

risk for coronary events [62]. From the preclinical study point of view, our findings provide strong evidence that nicotine can increase infarct size which is relevant to both the use of electronic cigarettes and tobacco smoking in humans.

There were some limitations of this study. Since nicotine can be metabolized to cotinine with a metabolic half-time of about 2 h, over the course of this experiment, cotinine levels on the order of 5–10 ng/ml blood would be present. A 10 ng/ml of cotinine concentrations increase in serum is associated with a 2% increase in myocardial ischemia in humans, so a contributory role for cotinine cannot be ruled out [63].

5. Conclusion

Our findings indicate that acute nicotine exposure can transiently elevate BP in healthy anesthetized SD rats and increase myocardial infarct size in an acute myocardial ischemia and reperfusion rat model. The increase in infarct size may reflect a more direct effect on the myocardium.

Declarations

Author contribution statement

L. Zhao: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

W. Dai; J. Carreno and J. Shi: Analyzed and interpreted the data.

M.T. Kleinman and R.A. Kloner: Conceived and designed the experiments; Wrote the paper.

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Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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