

Cardiac Effects of Cupping: Myocardial Infarction, Arrhythmias, Heart Rate and Mean Arterial Blood Pressure in the Rat Heart

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Abstract

This study was carried out to determine the effects of cupping on hemodynamic parameters, arrhythmias and infarct size (IS) after myocardial ischemic reperfusion injury in male rats. Rats were randomly subjected to dry or wet cupping. While dry cupping simply involved stimulation of the skin by suction, in wet cupping, scarification of the back skin was also carried out with a surgical blade and 0.5 ml blood was sucked out in each session. For ischemic reperfusion injury, rats were subjected to 30 min of left anterior descending coronary artery occlusion and 120 min of reperfusion. Our results show that cupping did not change the baseline heart rate or mean arterial blood pressure. Ischemic reperfusion injury caused an IS of $50 \pm 5\%$, whereas dry cupping, single and repeated wet cupping significantly reduced IS to $28 \pm 3\%$, $35 \pm 3\%$ and $22 \pm 2\%$ of area at risk, respectively. The rate of ischemic induced arrhythmias was significantly modified by wet cupping ($P < 0.05$). These results indicate for the first time in rats that cupping might be cardioprotective in the ischemic reperfusion injury model.

Key Words: cupping, nonischemic stimuli, cardioprotection

Introduction

Complementary and Alternative Medicine (CAM) is becoming more popular with the public and is gaining credibility within the biomedical health care community (14). Cupping, a traditional treatment, involves creating a vacuum over certain points on the skin which, in turn, generates a small visible hematoma. There are 2 main types of cupping: dry and wet cupping. While dry cupping simply involves stimulation of the skin by suction, wet cupping also uses laceration of the skin so that blood is extracted from dermal microcirculation (9). The after-effects of cupping often include erythema, edema and ecchymoses in a characteristic circular arrangement. These

bruises may take several days to several weeks to subside (18). In clinical studies, it is shown that wet cupping might exert effects on inflammation: the injury to the skin leads to release of β -endorphin and adrenocortical hormone into the circulation. Both hormones could be helpful in blocking inflammation in arthritis (1). This finding suggests that wet cupping exerts regulatory effects on the immune cells. It is also demonstrated that cupping therapy acts on the immune system *via* the central nervous system pathway by inducing β -endorphin and activation of the opioid system (1).

It is reported that an abdominal wall, or a shallow skin incision, elicits a preconditioning effect with early and late phases of protection against myocardial

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infarction (MI). The authors have named this phenomenon “remote preconditioning of trauma” (RPCT) (11). They also demonstrated that abdominal nociceptive stimulation (ANS) can produce cardioprotection similar to that of RPCT against MI (6). It is a reflex therapy where the nociceptive stimulus given in an area acts on another one (12).

To determine whether cupping, as a trauma or nociceptive stimulus, exerts protective effects against MI, a rat model of ischemic reperfusion (IR) injury was used in this study. The effects of cupping in animal models, according to our knowledge, have not yet been reported. This study was designed to evaluate the cardiac effects of cupping in an animal model for the first time.

Materials and Methods

Animals

Male Wistar rats weighing 250 to 300 g were housed under standard conditions with free access to food and water. The investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (1996), and was approved by the Animal Ethics Committee of Baqiyatallah University.

Surgical Procedure

All animals were anesthetized with sodium pentobarbital (30 mg/kg, ip, Sigma, St. Louis, MO, USA) and ketamine (40 mg/kg, ip, Alfasan, Holland) and supplemental doses (15 mg/kg, ip) of pentobarbital. Rectal temperature was continuously monitored and maintained at $37 \pm 0.5^\circ\text{C}$. The neck was opened with a ventral midline incision and a tracheotomy was performed. The rats were artificially ventilated with room air enriched with oxygen at a rate of 70 to 80 per min and a tidal volume of 1 ml/100 g to maintain blood PO_2 , PCO_2 and pH within the normal physiological range. A standard limb lead II electrocardiogram was monitored and recorded throughout the experiment. Catheters were inserted into the left carotid artery and tail vein (Angiocat 23; Suppa, Tehran, Iran) for monitoring of blood pressure and infusion of Evans blue solution, respectively. A left thoracotomy was performed in the fourth intercostal space, and pericardium was opened to expose the heart. A 6-0 silk suture was passed around the left anterior descending (LAD) coronary artery and the ends of it were threaded through a small polyethylene tube to form a snare. Following a stabilization period of 20 min, the artery was occluded for 30 min by clamping the snare against the surface of the heart. Ischemia was confirmed by regional cyanosis and ST

elevation in the electrocardiogram (ECG). Reperfusion was established by releasing the snare for 120 min (5).

Determination of Infarct Size (IS) and Area at Risk (AAR)

At the end of reperfusion, the LAD was re-occluded and 1 ml Evans Blue dye (2%, Sigma) was injected into the tail vein to identify the nonperfused area, also known as AAR, from the perfused area. The heart was excised, rinsed of excess blue dye, trimmed of atria and right ventricle, put in a matrix, wrapped in plastic foil and frozen at -20°C for 2 h. The left ventricle (LV) was cut into transverse slices of 2-mm thickness from the apex to the base. Tissue samples were then incubated in triphenyltetrazolium chloride 1% (TTC, Sigma) solution in isotonic phosphated buffer, pH 7.4, for 20 min at 37°C , and subsequently fixed in 10% formalin for 1 h. Viable tissues were stained red by TTC, whereas infarcted tissues were unstained (white). An image was obtained (canoscan LID25) from both sides of each slice and all calculations from one heart (using Image Tool Software) were averaged into one value for statistical analysis. IS was expressed as percentage of the AAR (IS/AAR).

Cupping

The protocol for performing cupping was as follows: after anesthesia with ketamine (50 mg/kg), the back of the animals was shaved with an electric razor. The skin was disinfected. For suction, a plastic cylinder 1.5 cm in diameter (as vacuum cup) was applied on the scruff and the air within the cup was rarefied by electromechanical suction (Shafabakhsh, Iran). The vacuum cup was kept in place for 3 min and repeated twice 1 min apart. In wet cupping, scarification (puncturing) of the skin was carried out by repeatedly puncturing it superficially with a surgical blade (number of incisions: 5 to 6) and 0.5 ml blood was sucked out. After removal of the cup, sterilized cotton was used to clear up the fluid and blood, and the region was then sterilized with alcohol cotton and kept in clear and dry with no covering. Immediately after wet cupping, lactated Ringer solution (2 ml/kg) was given ip. Cupped rats did not display discomfort or pain, moved freely about the cage, and consumed food and water immediately after recovering from anesthesia.

Experimental Protocols

Experiments were performed in the following groups of rats ($n = 7$ in each group): *Control*, 30-min ischemia and 120-min reperfusion (IR) (CO group);

Table 1. Hemodynamics parameters in the experimental groups

Groups	Baseline		Ischemia		Reperfusion	
	HR	MBP	HR	MBP	HR	MBP
CO	336 ± 18	124 ± 8	343 ± 17	105 ± 7*	356 ± 13	114 ± 8
Sham	337 ± 13	120 ± 4	340 ± 10	105 ± 3**	324 ± 8	107 ± 4
DC	350 ± 11	121 ± 6	370 ± 15	100 ± 6**	338 ± 23	100 ± 7
WC	378 ± 9	120 ± 3	383 ± 13	104 ± 5**	388 ± 14	116 ± 4
3WC	337 ± 12	120 ± 5	336 ± 13	100 ± 5**	348 ± 8	112 ± 5

Data are presented as means ± SEM. HR, heart rate; MBP, mean arterial blood pressure * $P < 0.05$, ** $P < 0.01$ compared with the baseline within the same group. CO, control group, DC, dry cupping, WC, wet cupping, 3WC, applying WC once weekly for 3 sessions.

dry cupping, twice suction without bloodletting 1 h before IR (DC group); *wet cupping*, twice suction with bloodletting 1 h before IR (WC group); *repeated wet cupping*, performing wet cupping once weekly for 3 sessions, then rats underwent IR 1 week after the last session (3WC group); *sham*, rats were followed by the same experimental procedure as the repeated wet cupping except for the following: vacuum cup was inserted on the skin and no suction was performed.

Assessment of Ventricular Arrhythmias

Ischemia-induced ventricular arrhythmias were determined in accordance with the Lambeth conventions (16): ventricular ectopic beat (VEB) as a discrete and identifiable premature QRS complex (premature with respect to the P wave), ventricular tachycardia (VT) as a run of four or more VEB at a rate faster than the resting sinus rate and ventricular fibrillation (VF) as a signal for which individual QRS deflection can no longer be distinguished from one another. Complex forms (bigeminy and salvos) were added to VEB count and not analyzed separately.

Statistical Analysis

All values are expressed as means ± SEM. Analysis of variance (ANOVA) was used to assess an overall difference among the groups for each of the variables. If ANOVA was significant, comparison with the control was performed by Tukey multiple comparison tests. $P \leq 0.05$ was considered to reflect a significant difference between control and experimental values (analysis was performed using SPSS for Windows, version 16).

Results

Hemodynamic Parameters

The hemodynamic data of this study are summa-

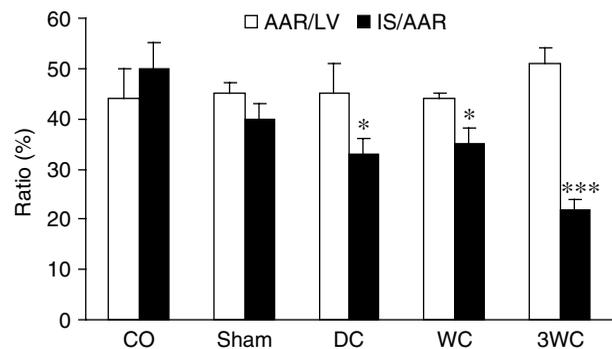


Fig. 1. Infarct size (IS) and area at risk (AAR) following 30-min ischemia and 120-min reperfusion in rats. LV, left ventricle; CO, control; DC, dry cupping; WC, wet cupping; 3WC, WC once weekly for 3 times. * $P < 0.05$ and *** $P < 0.001$ compared with the CO group.

rized in Table 1. There were no significant differences at baseline values for heart rate and mean arterial blood pressure (MBP) among the experimental groups. Ischemia caused a marked reduction in blood pressure that restored to the baseline level during the reperfusion period. Heart rate was increased during ischemia but it was not significant.

Infarct Size

As expected, myocardial ischemia and reperfusion induced infarct. AAR and IS following 30-min regional ischemia and 120-min reperfusion are shown in Fig. 1. There was no marked difference in AAR among the groups. The IS was significantly reduced in the DC (28 ± 3%), WC (35 ± 3%) and 3WC (22 ± 2%) groups compared with the CO group (50 ± 5%). There was no significant difference in IS between the CO and sham groups. No significant difference was observed between DC, WC and 3WC groups; however, the effect of repeated wet cupping was maximal with a reduction in IS of more than 50%.

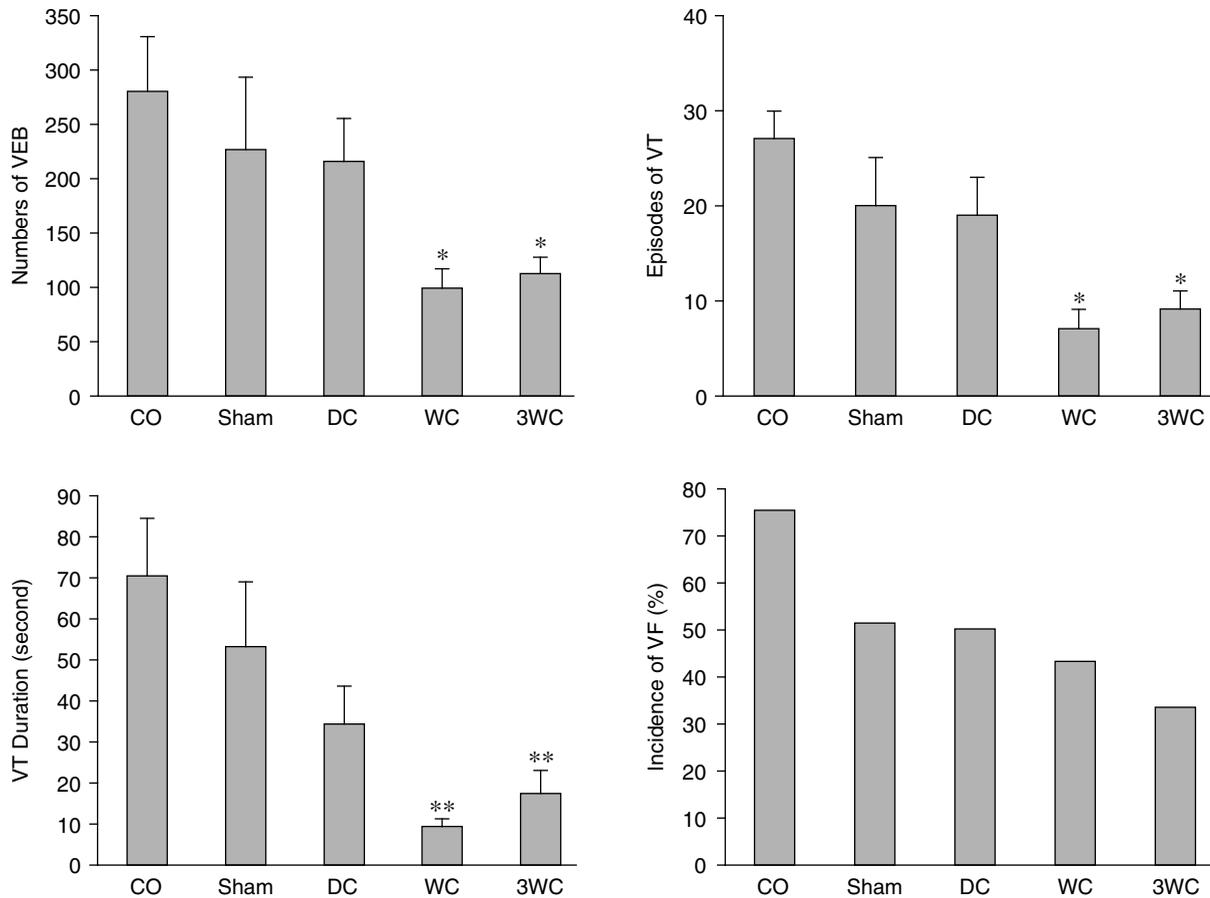


Fig. 2. VEB numbers, VT episodes and VT duration (as means \pm SEM) and the incidence of VF (as percent) during 30-min regional ischemia in rats. * $P < 0.05$; ** $P < 0.01$ versus the CO group. Abbreviations are as noted in Fig. 1 legend.

Ischemia-Induced Arrhythmias

The results of cupping on ischemia-induced arrhythmia are shown in Fig. 2. During 30-min ischemia, the number of VEB, VT episodes and VT duration in the CO group was 280 ± 51 , 27 ± 3 and 70 ± 14 s, respectively. The rate of these arrhythmias was significantly modified by treatment of the rats with wet cupping ($P < 0.05$). Despite the reduced rate of these arrhythmias in the DC group, there was no significant difference between the DC and CO groups. VF occurred in 75% of the CO group and lasted 22 ± 10 s, whilst VF incidence was reduced to 50%, 43% and 33% in DC, WC and 3WC, respectively. It should be noted that there were no considerable reperfusion-induced arrhythmias in the present work.

Discussion

The main findings of this study were: [1] cupping did not produce any significant effect on hemodynamic parameters *per se*, [2] dry and wet cupping caused a significant decrease in IS following 30-min ischemia

and 120-min of reperfusion, [3] wet cupping significantly reduced the VEBs numbers, VT episodes and duration during 30-min ischemia, [4] VF occurred in a lower percentage of rats treated with cupping, [5] wet cupping was more effective than dry cupping and [6] repeated wet cupping had more beneficial effects compared to single wet cupping.

Cupping is a physical treatment that involves the application of a vacuum to a localized area of the skin (18). In dry cupping, which pulls the skin into the cup without drawing blood, negative pressure acts on the skin and irritates subcutaneous muscles. In wet cupping, the skin is lacerated so that stagnant blood is drawn into the cup. It has been claimed that cupping, both dry and wet, drains excess fluids, loosens adhesions and lifts connective tissues, brings blood flow to stagnant skin and muscles, and stimulates the peripheral nervous system. In addition, cupping is said to reduce pain and high blood pressure and modulate neurohormone and immune systems (18). In one randomised clinical trial, the effectiveness of dry cupping on changes in cerebral vascular function was assessed in comparison with drug therapy. The

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results suggested significant effects in favour of cupping on vascular compliance and degree of vascular filling (7). In another study, acupuncture combined with cupping therapy could improve clinical symptoms, enhance tolerance of exercise, reduce angina frequency, duration and consumption of nitrates of the patients with stable exertional angina with fewer side effects (17).

It is reported that IS after *in vivo* IR was altered by nonischemic surgical trauma. Vascular surgery performed to catheterize the carotid artery increased IS, whereas abdominal wall or shallow skin incision resulted in a significantly decreased IS (11). To describe this nonischemic preconditioning phenomenon, the authors coined the term RPCT. They demonstrated that peripheral sensory nerve stimulation leads to activation of the cardiac sensory nerves *via* spinal nerves which, in part through calcitonine-gene related peptid (CGRP) release, stimulates cardiac sympathetic nerves. Release of NE and BK from the sympathetic nerves underlies cardioprotective response in the myocardium after RPCT. Direct activation of C sensory fibers in skin using topical capsaicin mimics the cardioprotective effect of RPCT, supporting that peripheral nociceptor stimulation has great clinical potentials (6).

In the present study, cupping resulted in a significant decrease in IS without any changes in the baseline heart rate and blood pressure. This effect was the most obvious in the 3WC group with more than 50% reduction. The suction and incision of skin during cupping causes trauma to superficial vessels and stimulates the peripheral nervous system. The mechanism of cupping might be the same as RPCT.

Like reperfusion, ischemia can cause lethal ventricular arrhythmias, including VT and VF (4). Meerson and Malyshev showed that adaptation to repeated stress reduces the incidence of ventricular fibrillation during a subsequent acute infarction (8). In the present work, we showed that pretreatment with wet cupping could reduce the incidence of ischemic arrhythmias including the numbers of VEBs, the episodes and duration of VT. A reduction in arrhythmia occurrence observed in the DC group including reduction of VT duration was not significant. If a larger sample size had been used in this investigation, better comparison in differences may be possible. Hence, further studies using larger groups of animals will be necessary to definitely access the incidence of ischemia-induced arrhythmia after cupping.

The cardioprotective effect obtained by a preceding cupping might be a result of a non-specific stress response. Release of NE from the cardiac sympathetic nerves following limb ischemia (10) and nociceptive stimulation of sensory nerve in the skin

of the abdomen (6) *per se* protect against both ischemia and reperfusion arrhythmias (2, 3). Although we did not evaluate NE release following cupping, it appears that cupping, as in any stress, increases NE release from cardiac sympathetic nerves. In general, signalling from one tissue to another may be achieved by humoral or neural pathways.

According to our knowledge, the effect of cupping in animal models has not studied yet. This is the first report in rat. The literature data and clinical studies mainly refer to the effects of acupuncture. The data clearly show that acupuncture has therapeutic effects for individuals with hypertension, myocardial ischemia and certain arrhythmias (15). It is documented that cupping should be capable of stimulating individual acupuncture points (13).

This study has some limitations: the phenomenon of increased tolerance to myocardial ischemia following cupping was only demonstrated in rats and it is not proven that it may have similar clinical effects in humans. Furthermore, the present study did not provide information on protection of cardiac functions, but it demonstrated protection against IS and arrhythmias. In conclusion, this study shows for the first time that cupping might be cardioprotective in ischemic reperfusion injury model of rats.

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