Anisodamine hydrobromide ameliorated cardiac damage after resuscitation

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Research Article

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Abstract

Background: The microcirculation is correlated with the prognosis of patients with cardiac arrest and changes after resuscitation.

Methods: This study was to study effects of anisodamine hydrobromide (AH) on microcirculation and explore its potential mechanisms. Twenty-four pigs were randomly grouped into three groups (n = 8): Sham, Saline and AH group. After pigs were anesthetized, intubated and mechanically ventilated, ventricular fibrillation (VF) was induced by electrical stimulation. After 8 mins, cardiopulmonary resuscitation (CPR) was given to the restoration of spontaneous circulation (ROSC). Arteriovenous blood was collected at baseline and 0, 1, 2, 4, and 6 hrs after ROSC to measure blood gas and cytokines. Perfused vessel density (PVD) and microvascular flow index (MFI) were measured to reflect the microcirculation. Continuous cardiac output (CO) and global ejection fraction (GEF) were measured to indicate hemodynamics.

Results: Compared with Sham group, PVD and MFI in the intestines and the sublingual regions decreased significantly after resuscitation. The microcirculation recovered faster in the AH group than the SA group. The decrease of intestinal microcirculatory blood flow was closely related to the decrease of sublingual microcirculatory blood flow. The cardiac function was impaired after resuscitation, and a decrease in the ratio of cytokine IFN-γ/IL-4 suggested the immune imbalance. The microcirculation changes in sublingual regions were closely related to the changes in intestines.

Conclusion: AH could improve the immune imbalance after resuscitation and was beneficial to the recovery of cardiac function.

Background

Although the success rate of resuscitation from cardiac arrest can reach 50% after the implementation of high-quality cardiopulmonary resuscitation (CPR), only 5–15% of cases can survive to discharge due to the poor prognosis [1]. The syndrome shown by myocardial dysfunction, cerebral injury and systemic organic ischemia-reperfusion injury which could be illustrated as ischemia/reperfusion injury (IRI) and due to the systemic inflammatory responses is the core characteristic of post-resuscitation syndrome [2]. Studies have confirmed that the intestines may be the most sensitive parts to IRI. Cardiac arrest lead to a continuous decrease in intestinal blood flow and an increase in intestinal permeability, subsequently triggering systemic inflammatory responses, which could be the underlying mechanism to cause the sepsis [3–5]. Inflammatory cytokines are elevated after CPR on the occurrence of sepsis. However, it is still unclear whether the post-resuscitation syndrome will affect intestinal circulatory function. The microcirculation is important in the treatment for patients in need of critical care because it plays a pivotal role in the oxygen supply and the nutritional supplementation of tissues [6].

It has been reported that there is an inconsistency between systemic blood flow and tissue perfusion in patients with cardiac arrest [7, 8], but the microcirculation is correlated with the prognosis of patients [7,

9–11]. Therefore, microcirculatory dysfunction could be decisive in the prognosis of circulatory failure [12, 13]. Microcirculation varies greatly among different organs, especially under low-flow conditions [3, 8, 14]. It is easy to detect the microcirculation in sublingual region as a perfect part for evaluating microcirculation [15]. However, it is unclear whether it can fully reflect the visceral microcirculation.

Anisodamine, a commonly used anti-shock drug, is an anticholinergic drug extracted from Chinese herbal medicine with many beneficial effects. Anisodamine was reported to increase intestinal perfusion during the electric shock [16], but there is few studies on its effects on intestinal mucosal blood flow and metabolism. In this study, the intestinal and sublingual microcirculation was measured in CPR pig model, and changes in hemodynamic indicators and inflammatory cytokines were detected to identify the relationship between microcirculatory changes and inflammatory responses. Furthermore, anisodamine hydrobromide (AH) was administered to evaluate its protective effects.

Methods

Chemicals and Reagents

Interleukin 4 (IL-4) and interferon gamma (IFN-γ) assay kits were purchased from Sunbio Biotech Co. Ltd. (Beijing, China). AH was purchased from the National Institutes for Food and Drug Control (Beijing, China) with more than 99% purity. AH was dissolved in saline for treatment.

Experimental Procedures and Treatment

Twenty-four male Beijing white pigs (12–14 months old, 30 ± 2 kg) were purchased from the Institute of Zoology, Chinese Academy of Sciences (Beijing, China). All animals were housed in the specific pathogen-free environment. Pigs had free access to food and water during the experimental period. Pigs were fasted overnight but allowed free access to water before the experiment. All animal experiments were performed following the Chinese legislation on the use and care of laboratory animals and approved by the Institutional Animal Care and Use Committee of Capital Medical University (IACUC protocol No. 2019–2256, Beijing, 9/23/2019). We have reported our study according to the relevant Equator network guideline and followed the Animal pre-clinical research guideline (ARRIVE). Effects were made to minimize the number of animals utilized and to decrease their suffering.

After an intramuscular injection of midazolam (0.5 mg/kg, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), anesthesia was induced by the intravenous injection of propofol (1.0 mg/kg, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) and maintained with intravenous infusion of pentobarbital (8 mg/kg/h, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China). Heart rate and electrocardiogram measurements were monitored using a four-channel physical recorder (BL-420F Data Acquisition& Analysis System; TME Technology Co. Ltd, Chengdu, China). A cuffed 6.5 mm cannula was advanced into the trachea. Pigs were ventilated with a volume-controlled ventilator (Servo 900C; Siemens, Germany) with a fraction of inspiration O_2 (FiO_2) at 0.35 and a respiratory frequency of 12 breaths/min using a tidal volume of 15 ml/kg. The aortic pressure was measured by an angiographic catheter

inserting from the femoral artery into the aortic arch. All hemodynamic parameters were monitored by the M1165 system (Hewlett-Packard, Palo Alto, CA, USA).

Prior to the induction of cardiac arrest, pigs were allowed to equilibrate for 30 mins to reach the stable level after anesthesia. The conductor of temporary pacemaker was inserted into the right ventricle through the right sheath and connected to an electrical stimulator (GY-600A; Kaifeng Huanan Equipment Co., Ltd., China) with the S1S2 mode (300/200 ms, 40 V), which provided the continuous electrical stimulation with a proportion of 8:1 and a step length of 10 ms, until ventricular fibrillation (VF) occurred [17] and the mean aortic pressure (MAP) suddenly dropped to zero.

Ventilation was withheld for 8 mins after the onset of VF. Manual CPR was then conducted at a frequency of 100 compressions/min with ventilation at FiO_2 of 100% and a compression-to-ventilation ratio of 30:2. The quality of chest compressions was controlled by a HeartStart MRx Monitor/Defibrillator with Q-CPR (Philips Medical Systems, the Netherlands). If the spontaneous circulation was not restored, defibrillation was attempted with the mode of 150 J.

ROSC was defined by the systolic blood pressure above 50 mmHg for more than 10 min. If spontaneous circulation was not restored within 30 min, the pig was considered dead [18]. Immediately after successful CPR, pigs were randomly divided into 2 groups (n = 8): Saline and AH. Saline or AH (4 mg/kg) was administered via central venous injection. The same procedures without VF initiation were conducted in the Sham group. At the end of the study, pigs were euthanized with an overdose of pentobarbital (150 mg/kg) via the femoral artery.

Outcome Measurement

Both the real-time mean arterial blood pressure (ABP) and central venous pressure (CVP) were measured. Continuous cardiac output (CO) and global ejection fraction (GEF) were checked through a pulmonary artery catheter.

Arterial Blood Gas

Arterial blood was collected at baseline and 0, 1, 2, 4, and 6 hr after ROSC and arterial blood gas was measure using the blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Lexington, MAs).

Measurement of Cytokines

Serum IL-4 and IFN-γ were measured using enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's instructions (Sunbio Biotech Co. Ltd., China).

Hematoxylin and Eosin (H&E) Staining

After sacrifice, hearts were harvested and fixed with 4% formalin for 24 h and embedded in paraffin. 5-μm serial sections were stained with H&E. Histological analysis was performed by an experienced pathologist

blinded to the experimental groups using an Olympus BX60 microscope (Tokyo, Japan).

Terminal Deoxynucleotidyl Transferase dUTP Nick-end Labeling (TUNEL) Staining

TUNEL staining was performed with an in situ cell death detection kit according to the manufacturer's instructions. Slides were mounted in DAPI containing Vectashield (Vector) and visualized with an Olympus BX60 microscope (Tokyo, Japan).

Western Blot

The heart tissues were harvested, washed in PBS three times and then homogenized in lysis buffer with protease inhibitors. Protein concentration was determined by bicinchoninic acid assay assay. Samples with 50 μg protein were run on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, and proteins were electrotransferred to PVDF membranes. The membranes were blocked with 5% non-fat milk in PBS for 1 hr and then incubated with primary antibodies at 4 ^{O}C overnight. After washing, blots were washed with PBS, and then incubated with the horseradish peroxidase-conjugated secondary antibody for 1 hr. Signals were detected using an ECL kit (Bio-Rad, Hercules, CA, USA).

Microcirculation

A video microscope, namely the sidestream dark field imaging (MicroScan, Microvision Medical, Amsterdam, Netherlands) was used for the observation of the microcirculation. Shortly, a handheld video microscope emits stroboscopic green light, which is absorbed by the erythrocytic hemoglobin and released back immediately. The images of the erythrocytes moving in the micro-vessels are transmitted to the camera through the microscope, thereby obtaining a non-invasive real-time image of the microcirculation. The microcirculation of the experimental animals at 0, 1, 2, 4, and 6 hr after ROSC was recorded by manual operation. In order to observe the intestinal microcirculation, 2–3 cm segment of jejunum has been isolated. Additionally, it has been coated with gauze soaked in warm saline. The mesentery on the serosal side was used as an observing region to evaluate intestinal microcirculation. After the observation, the intestine was back to the peritoneal cavity. The abdominal wall was sutured, and the skin incision was closed through the mode of a wound clip. The images were stored and analyzed offline using AVA-Automated Vascular Analysis 3.1 (Microvision Medical) to obtain Perfused vessel density (PVD) and microvascular flow index (MFI) parameters. These parameters were calculated for microvessels (≤ 20 μm in diameter). For PVD, microvessels were classified as either perfused (hyperdynamic, continuous or slow blood flow in the vessels) or non-perfused (intermittent or no blood flow) based on the microcirculation. For the quantification of MFI, the images were put into four parts. In addition, the microcirculatory condition was shown in a scheduled scale.

Statistical Analysis

Data were presented as mean ± SD and analyzed by SPSS 17.0 (SPSS Inc, Chicago, IL, USA). Data between two groups were compared using the Student's t-test; data among multiple groups were

compared using one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant difference.

Results

AH Treatment Promoted the Recovery of Cardiac Function

It could be seen that none of distinct variation expressed in body weight, hemodynamic indexes and blood parameters at baseline between groups (Table 1). All animals induced by VF have been resuscitated in a good condition. There was none variation of defibrillation and the time of resuscitation between SA and AH group. With the comparative analysis of baseline group, the cardiac function has been seriously impaired after CPR. The recovery was gradual with the duration, and the cardiac function in AH group recovered faster than SA group (Fig. 1) (Table 2).

Table 1
Baseline characteristics

	Sham (n = 8)	**Saline (n = 8)**	**AH (n = 8)**
Weight (kg)	23.75 ± 0.96	24.13 ± 1.96	24.0 ± 1.41
Heart rate (bpm)	101.75 ± 5.85	102.38.75 ± 6.63	101.63.5 ± 5.53
MAP (mmHg)	99.50 ± 11.94	100.70 ± 12.38	99.45 ± 11.63
CO (L/min)	3.60 ± 0.22	3.65 ± 0.28	3.68 ± 0.28
pH	7.41 ± 0.06	7.38 ± 0.09	7.34 ± 0.09
Lactate (mmol/L)	2.15 ± 0.44	2.17 ± 0.57	2.14 ± 0.55
MAP: mean arterial pressure; CO: cardiac output			

Table 2
Characteristics at 6 hr post-resuscitation.

Group	Sham (n = 8)	Saline (n = 8)	AH (n = 8)
Heart rate (bpm)	102.5 ± 6.61	128.25 ± 8.83##	110.0 ± 7.80**
MAP (mmHg)	90.25 ± 4.28	105.38 ± 6.22#	97.58 ± 5.91*
CO (L/min)	3.64 ± 0.17	2.77 ± 0.23#	3.34 ± 0.33**
pH	7.38 ± 0.03	7.05 ± 0.13#	7.28 ± 0.12*
Lactate (mmol/L)	1.98 ± 0.31	4.79 ± 0.41##	3.11 ± 0.38*
#$p < 0.05$, ##$p < 0.05$ vs. Sham group; *$p < 0.05$, **$p < 0.01$ vs. SA group.			

AH Treatment Decreased Myocardial Tissue Damage

After treatment, hearts were harvested for histopathological analysis. Obvious pathological changes were observed in the saline group. However, myocardial tissue damage was markedly ameliorated after AH treatment. TUNEL staining of myocardial tissues showed that apoptosis significantly decreased after AH treatment. Moreover, Pro-apoptotic protein Bax expression decreased, but anti-apoptotic protein Bcl-2 increased after AH treatment (Fig. 2).

AH Treatment Improved the Microcirculation

After resuscitation, PVD and MFI decreased significantly in the intestines and the sublingual region, comparing to the Sham group. Afterwards, PVD and MFI gradually recovered with time. However, these microcirculatory indexes recovered faster in the AH group than the SA group. At 2, 4 and 6 h after resuscitation, there were significant differences of PVD and MFI in both intestines and the sublingual region between two groups. Moreover, the microcirculatory indexes (PVD and MFI) had a strong correlation between the intestines and the sublingual regions (Figs. 3 and 4).

AH Treatment Inhibited the Transformation of Th1 to Th2

IL-4 and IFN-γ levels were measured in serum. Results showed that IL-4 secretion significantly increased after ROSC, but IFN-γ level significantly decreased. However, AH treatment significantly decreased the IL-4 level and increased the IFN-γ level (Fig. 5). These data indicated that the ROSC promoted Th1 to Th2 transformation, but was largely inhibited by AH treatment.

Discussion

This study demonstrated that the intestinal and sublingual microcirculatory blood flow declined dramatically after cardiac arrest and successful CPR. The correlation analysis between the microcirculatory indexes and the hemodynamic indexes showed that intestinal microcirculatory dysfunction has a good relationship with the serious situation of post-resuscitation syndrome. In addition, variations in sublingual microcirculation have been key to the changes in the intestinal microcirculation. AH treatment can help the recovery of hemodynamic function and the maintenance of immune balance, and had protective effects after resuscitation.

The sublingual region is the easiest part for the measurement of microcirculation. Previously, the sublingual microcirculation has been regarded as a surrogate index for visceral blood flow. Studies have proposed that non-invasive sublingual CO_2 detection can be used as an alternative measurement of gastric pressure in case of microcirculatory disturbance [19–22]. This study showed that the sublingual microcirculation had a similar performance to the intestinal microcirculation in the early post-resuscitation stage by the visualized method. This result was consistent not only with previous studies on microcirculatory disturbance, but also with studies on endotoxin and septic shock, indicating that the microcirculatory changes in severity and process are similar between sublingual and intestinal region [23–25]. Nevertheless, there are still some controversies. A clinical study [26] proposed that the microcirculatory changes in the two parts were not parallel on the first day after the occurrence of sepsis. Another animal study [27] showed that the correlation of the microcirculation in the two parts disappeared with time. It is speculated that some of the treatment measures given in the above two studies may affect the intestinal microcirculation, leading to different conclusions of these two studies. In addition, sepsis itself is highly heterogeneous [28].

The abnormal manifestations after resuscitation resemble those of sepsis, and cardiac dysfunction shall be one of the main features. The process of the cardiac function after CPR causes a decrease in visceral blood flow. However, the changes in microcirculation are not exactly equivalent to the variations expressed in the blood flow. In addition, there are various points between visceral organs, in particular notes, in the situation of low flow [29]. The correlation between the microcirculation and the cardiac function in the early post-resuscitation stage was confirmed in this study. The parameters of the intestinal microcirculation after resuscitation showed a tendency to recover, which was considered to have a certain relationship with the body's automatic regulation and the treatment measures. Actually, it could be seen that the control of microcirculatory blood flow concerning the intestinal wall shall be too complex.

In terms of both systemic factors and local factors could make contribution to the intestinal microcirculation, including but not limited to inflammatory cytokines, vasomotor function. In this study, the parameters of sublingual and intestinal microcirculation and the hemodynamic parameters indicated the cardiac function of animals was better in the AH group than SA group, suggesting that AH can help improve the microcirculation and the cardiac function, and has protective effects after resuscitation.

The integrity of the intestinal mucosal barrier takes a proactive function in the progress of systemic inflammatory response syndrome sepsis as well as multiple organ failures. Th1 lymphocytes produce pro-inflammatory cytokines, such as IFN-γ, which mainly contributes to cell-mediated immune responses. Th2 lymphocytes secrete anti-inflammatory cytokines, such as IL-4, for host defence against invasion by exogenous pathogens [30, 31]. The balance between Th1 and Th2 cells plays a vital role in maintaining normal immune function. In previous ROSC studies on cardiac arrest models, abnormal Th1/Th2 ratios were found in spleen, lung and myocardium [32, 33]. This study showed that after resuscitation, the level of serum IFN-γ continued to decrease, meanwhile, the level of serum IL-4 increased significantly. These data were consistent with previous observations in individuals and animal models of cardiac arrest undergoing CPR [34]. AH treatment significantly decreased the IL-4 level and increased the IFN-γ level, indicating that AH alleviated the transformation of Th1 to Th2, and the immune imbalance induced by IRI was improved.

Clinically, intestinal injury after cardiac arrest may be underestimated after successful CPR due to non-specific and delayed manifestations, although it may lead to fatal complications [35]. It is not clinically feasible to observe the intestinal microcirculation directly in vivo, so the early detection is difficult.

Conclusions

This study showed the variations in the process sublingual microcirculation after resuscitation could indicate the variations in the process of intestinal microcirculation. In addition, to some certain extent, the changes in the cardiac function, suggest that the sublingual microcirculation may be a new alternative for bedside monitoring of post-resuscitation patients.

Abbreviations

arterial blood pressure (ABP); anisodamine hydrobromide (AH); cardiac output (CO);

cardiopulmonary resuscitation (CPR); central venous pressure (CVP); global ejection fraction (GEF); hematoxylin and eosin (H&E); interferon gamma (IFN-γ); interleukin 4 (IL-4); ischemia/reperfusion injury (IRI); mean aortic pressure (MAP); microvascular flow index (MFI); perfused vessel density (PVD); restoration of spontaneous circulation (ROSC); terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL); ventricular fibrillation (VF)

Declarations

Ethical approval and consent to participate

All animal procedures were approved by the Institutional Animal Care and Use Committee of Capital Medical University.

Consent to publication

Not Applicable

Availability of data and materials

The data are available from the corresponding author upon reasonable request.

Competing interests

Guijuan Dong declares she has no conflict of interests.

Shubin Guo declares he has no conflict of interests.

Funding

Not Applicable

Authors' contributions

Study concepts and design: SBG

Experimental studies: GJD, SBG

Data analysis: GJD

Manuscript preparation: GJD, SBG

All authors have read and approved the manuscript.

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Figures

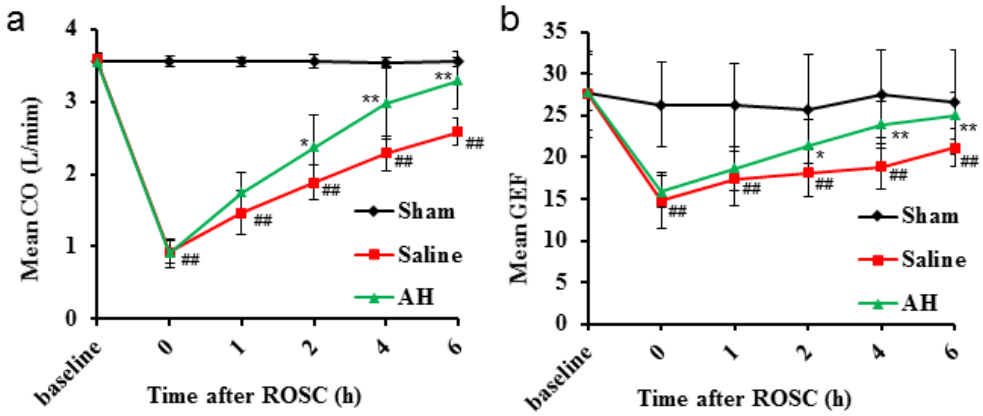

Figure 1

AH treatment promoted the recovery of cardiac function. CO and GEF were recorded at 0, 1, 2, 4, and 6 hr after ROSC. CO and GEF decreased significantly, comparing to the Sham group. However, CO and GEF recovered faster in the AH group than the SA group (a, b). Data were expressed as mean ± SD (n = 8). $^{\#\#}p < 0.01$ vs. Sham group; $^{*}p < 0.01$, $^{**}p < 0.01$ vs. Saline group.

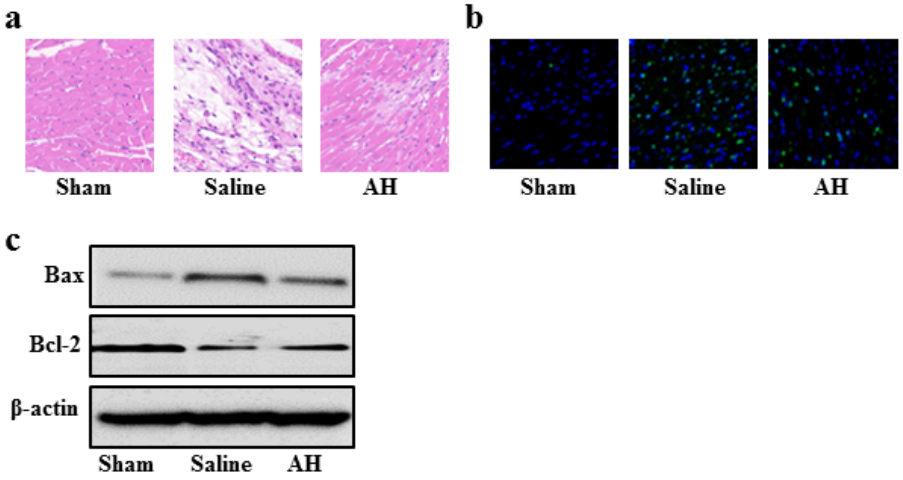

Figure 2

AH treatment decreased myocardial tissue damage. AH treatment ameliorated myocardial tissue damage shown by H&E staining (a). AH treatment also decreased apoptosis in myocardial tissues, shown by TUNEL staining (b). Furthermore, AH treatment decreased pro-apoptotic protein Bax expression, whereas increasing anti-apoptotic protein Bcl-2 expression (c).

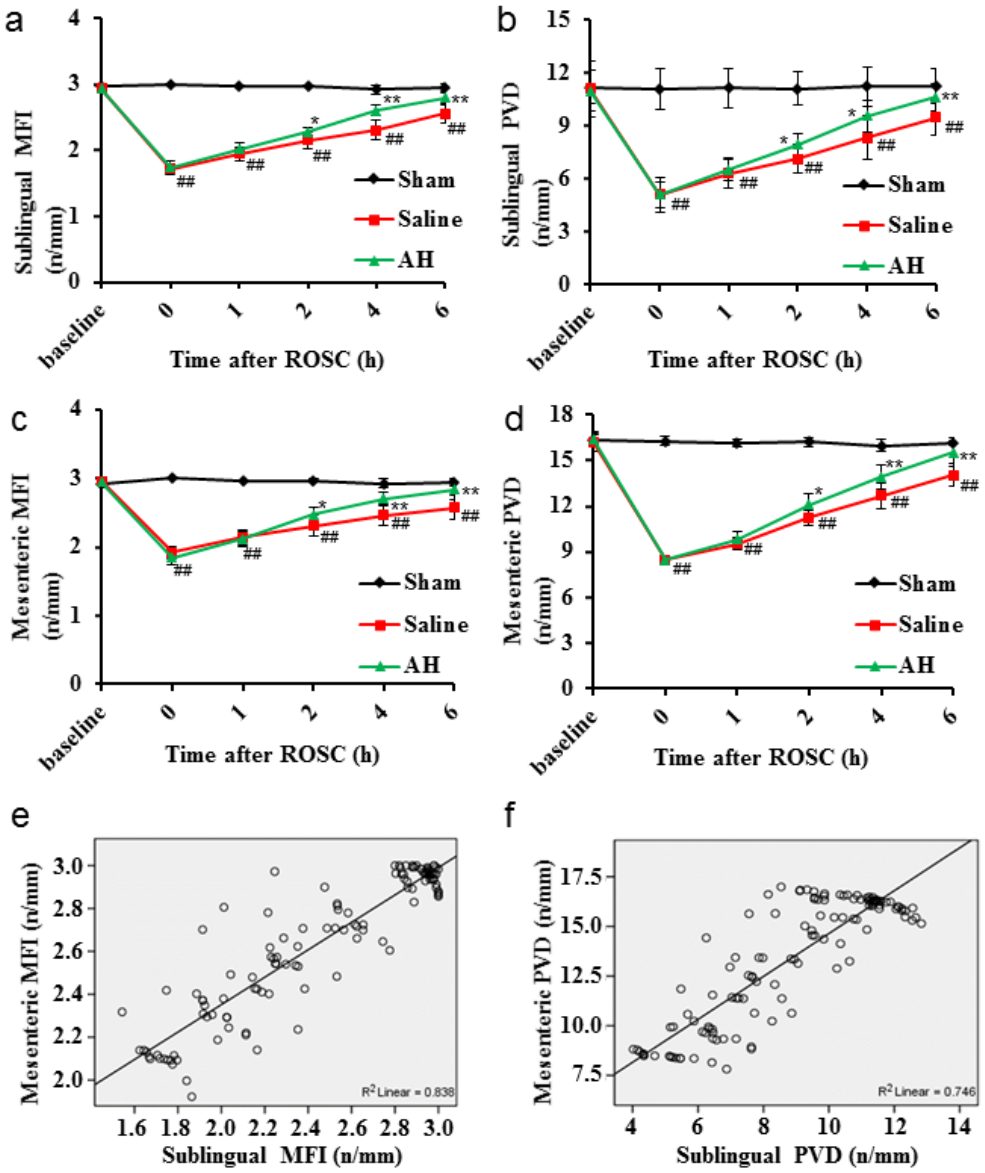

Figure 3

AH treatment improved the microcirculation. PVD and MFI in the intestines and the sublingual regions were recorded at 0, 1, 2, 4, and 6 hr after ROSC. PVD and MFI decreased significantly, comparing to the Sham group. However, these microcirculatory indexes recovered faster in the AH group than the SA group (a-d). Moreover, the microcirculatory indexes (PVD and MFI) had a strong correlation between the

intestines and the sublingual regions (e, f). Data were expressed as mean ± SD (n = 8). $^{\#\#}p < 0.01$ vs. Sham group; $^{*}p < 0.01$, $^{**}p < 0.01$ vs. Saline group.

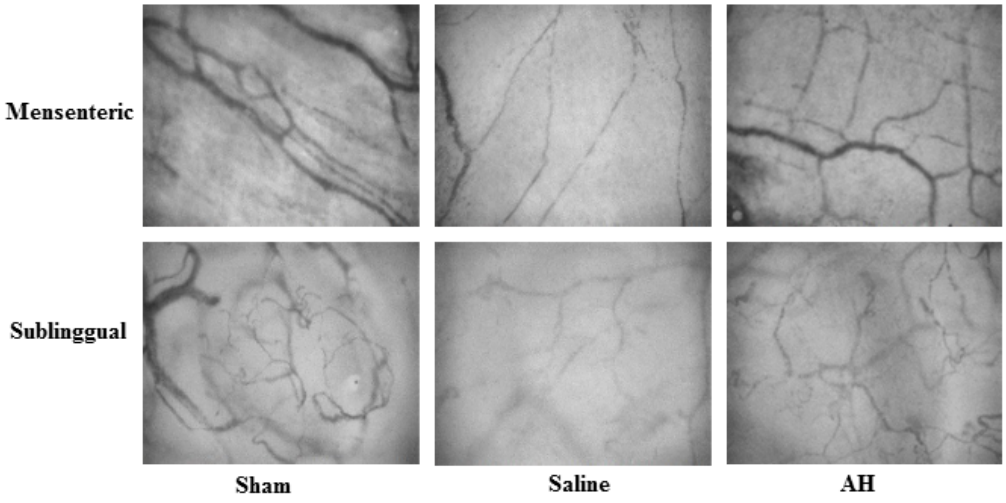

Figure 4

AH treatment improved the microcirculation. Photos of microcirculation in the intestines and the sublingual regions were taken 6 hr after ROSC. AH treatment significantly improved the microcirculation both in the intestines and the sublingual regions.

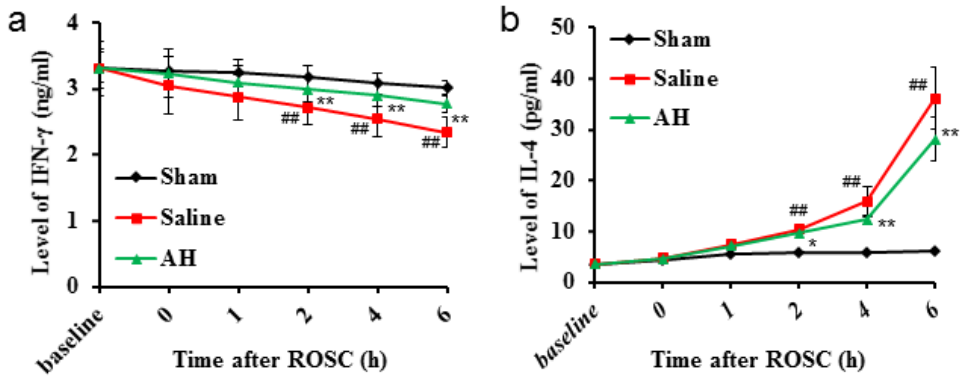

Figure 5

AH treatment inhibited Th1-Th2 transformation in the blood. Blood samples were collected at baseline, 0, 1, 2, 4 and 6 h after ROSC. Compared with the Saline group, AH treatment significantly increased IFN-γ level, and decreased IL-4 level in serum. Data were expressed as mean ± SD (n = 8). $^{\#\#}p < 0.01$ vs. Sham group; $^{*}p < 0.01$, $^{**}p < 0.01$ vs. Saline group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supp1.png
- ARRIVEGuidelinesChecklist.pdf