

Research Article:

## Nitroglycerin Improves Microcirculation During and After Cardiopulmonary Resuscitation in a Porcine Model of Cardiac Arrest

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### Abstract

#### 1. Objective

We have previously demonstrated that vasopressors administered during cardiopulmonary resuscitation (CPR) significantly decrease microcirculatory flow. In the present study, we investigate the effect of nitroglycerin (NTG) on the macro and microcirculation during and after CPR in a porcine model of cardiac arrest (CA). We hypothesize that NTG improves microcirculation without undesirable effects on the macrocirculation during and after resuscitation.

#### 2. Methods

Ten male domestic pigs weighing  $40 \pm 2$  kg were utilized. Ventricular fibrillation was electrically induced and untreated for 5 min. The animals were then randomized to receive NTG (N group, n=5) or saline (C group, n=5). Coincident with the start of CPR, either NTG (5  $\mu$ g/kg) or saline was injected into the right atrium. Defibrillation was attempted by a single 150 J shock after 5 min of CPR. Hemodynamics were recorded continuously and sublingual microcirculation was assessed with the orthogonal polarization spectral (OPS) at baseline (BL), 1, and 5 min of CPR and 1 and 5 min after resuscitation.

#### 3. Results

All animals were resuscitated successfully. There was no difference in coronary perfusion pressure (CPP) and end-tidal carbon dioxide (ETCO<sub>2</sub>) between the two groups. Sublingual perfused vessel density (PVD) and microcirculatory flow index (MFI) were greater in NTG treated animals than those of control animals during and after CPR.

#### 4. Conclusion

Administration of nitroglycerin at the onset of CPR improves microcirculation without undesirable effects on macrocirculation during and after resuscitation.

**Keywords:** Nitroglycerin; Microcirculation; Cardiopulmonary resuscitation; Macrocirculation; Epinephrine; Ventricular fibrillation

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## Introduction

The primary goal of resuscitation is to restore critical levels of blood flow to vital organs such that spontaneous circulation can be restored. Epinephrine is a potent vasopressor agent and is recommended by the American Heart Association (AHA) as the routine intervention during CPR. Epinephrine increases coronary and cerebral perfusion pressure and may favour initial resuscitation [1-4]. Primary vasopressor efficacy of epinephrine is due to its alpha-1 and alpha-2 adrenergic effects. However it increases the severity of cerebral ischaemia because of a reduction of cerebral microcirculatory flow [5-8].

NTG is a powerful vasodilator. It reduces venous return and cardiac preload [9], and therefore decreases ventricular filling pressure and wall stress [10-12]. The reduction in wall tension decreases the subendocardial resistance to blood flow. Studies have demonstrated that NTG significantly reduces cardiac filling pressures and improves microvascular perfusion in patients admitted for acute heart failure or myocardial ischemia reperfusion injury [13,14].

We have previously demonstrated that epinephrine administered during CPR significantly decreases microcirculatory blood flow [5-7, 15]. In this study, we investigate the effects of NTG on the macro and microcirculation during and after CPR in a porcine model of CA. We hypothesize that NTG would improve microcirculation without undesirable effects on the macrocirculation during and after resuscitation.

## Materials and Methods

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the

National Institutes of Health (8th edition; Washington, DC, National Academic Press, 2011). The protocol was approved by the Institutional Animal Care and Use Committee of the Weil Institute of Critical Care Medicine. The approval number is IACUC-TLECCM-P1501.

### 1. Animal preparation

Ten male domestic pigs weighing  $40 \pm 2$  kg were fasted overnight except for free access to water. Anaesthesia was initiated by intramuscular injection of Midazolam (0.4 mg/kg), followed by ear vein injection with sodium pentobarbital (30 mg/kg).

Additional doses of sodium pentobarbital (8 mg/kg) were administered at intervals of approximately 1 hour or when required to maintain anesthesia. A cuffed endotracheal tube was advanced into the trachea. Animals were mechanically ventilated with a volume controlled ventilator (Model WATO EX-65, MINDRAY, Shenzhen, China) with a tidal volume of 12 ml/kg, peak flow of 40 L/min, and FiO<sub>2</sub> of 0.21. ETCO<sub>2</sub> was monitored with an infrared capnometer (Model BeneView T5, MINDRAY, Shenzhen, China). Respiratory frequency was adjusted to maintain ETCO<sub>2</sub> between 35 and 40mmHg before CA. For recording the frontal plane electrocardiogram (ECG), three adhesive electrodes were applied to shaved skin of the proximal right upper and left upper-and-lower limbs. ECG was also conducted with a Mindray monitor (Model BeneView T5, MINDRAY, Shenzhen, China). The measurement of aortic pressure and collection of blood samples was obtained by use of a 5 F Swan-Ganz catheter (Edwards Lifesciences LLC, Irvine, CA) advanced from the right femoral artery into the thoracic aorta. Right atrial pressure (RAP) and core body temperature, were measured using a 7 F Swan-Ganz catheter (Edwards Lifesciences LLC, Irvine, CA) advanced from the

right femoral vein and directed into the right atrium. Both catheters were flushed intermittently with saline containing 5 IU bovine heparin per ml. Induction of ventricular fibrillation (VF), was achieved by a 5 F pacing catheter (EP Technologies Inc., Mountain View, CA) advanced from the right external jugular vein into the right ventricle. The position of all catheters was confirmed by characteristic pressure morphology. A pair of shock electrodes (Adult Electrodes with HVP Gel, Zoll Medical Corporation, Chelmsford, MA) connected with an external biphasic defibrillator (Model E-Series, Zoll Medical Corporation, Chelmsford, MA) were attached in a lateral-to-lateral configuration, with the positive electrode placed at the right anterolateral thorax and the negative electrode placed at the left anterolateral thorax with the heart directly interposed between them. The piston of a compressor known as the smart chest compressor (SCC) (SCCTM, Shanghai 3F Electronics Co., Ltd., China) was positioned midline at the level of the fifth intercostal space. Body temperature was maintained at  $37.5 \pm 0.5^\circ \text{C}$  with the aid of a cooling/warming blanket (HGT-200 II, Zhuhai Hokai Medical Instruments Co., Ltd, China) throughout the experiment.

Sublingual microcirculation, was measured with a CapiScope Handheld Video Capillaroscope (HVCS) system (KK Technology, Honiton, England) using a  $5\times$  imaging objective. Three discrete fields were captured with precaution to minimize motion artifacts and microvascular images were recorded.

## 2. Experimental procedures

Fifteen minutes prior to inducing CA, baseline measurements were obtained, which included microcirculation images. The animal was then randomized by the Sealed Envelope Method to receive NTG (N group) or saline (C group). Cardiac

arrest, due to VF, was induced by a 1mA alternating current through a 5 F pacing catheter that was placed into the right ventricular cavity. Mechanical ventilation was discontinued after the onset of VF. Prior to initiating the resuscitation procedure, the pacing catheter was withdrawn to avoid heart injury during chest compression. After 5 min of untreated VF, CPR was performed. The SCC was programmed to provide 100 compressions-per-minute. The compression depth was adjusted to decrease the anterior-posterior diameter of the chest by 25%. Coincident with the start of precordial compression, all animals were mechanically ventilated with a tidal volume of 12 mL/kg and  $\text{FiO}_2$  of 1.0, with a rate of 10 breaths-per-minute. Coincident with the onset of CPR, NTG (5  $\mu\text{g}/\text{kg}$ ) or the same volume of saline was administered into the right atrium through the femoral vein. During the time of CPR, microcirculation images were obtained at 1 and 5 min of CPR. After 5 min of CPR, defibrillation was attempted with a single 150 J biphasic electrical shock delivered between the lateral-to-lateral electrodes with a defibrillator. Microcirculation images were continuously obtained after delivering shock. If an organized rhythm with a mean aortic pressure of  $>50 \text{ mmHg}$  persisted for an interval of 5 min or more, the animal was regarded as successfully resuscitated; this is also known as return of spontaneous circulation (ROSC). If ROSC was not achieved, chest compression and ventilation were immediately continued for 2 min before a subsequent single defibrillation attempt. The procedure was repeated for a maximum of 3 cycles. If ROSC was not achieved, the resuscitation manoeuvre was terminated. After every experiment, microcirculation images at 1 and 5 min after resuscitation were obtained for further analysis. After a period of 5 min, post-resuscitation measurements were completed. All catheters were removed and wounds were surgically sutured. Animals

were euthanized painlessly by intravenous injection of 150mg/kg pentobarbital. An autopsy was routinely performed to identify any injuries to the bony thorax, thoracic, or abdominal viscera.

### 3. Measurements

Hemodynamics, ETCO<sub>2</sub>, and ECG were continuously measured and recorded on aPC-based data acquisition system, supported by CODAS/WINDAQ hardware/software (Computer Acquisition System, Cambridge, MA). CPP was digitally computed from the differences in time-coincident diastolic aortic and right atrial pressure and displayed in real time. Acute ECG changes after CPR and defibrillation shocks were measured by continuous ECG recordings. Arterial blood gases including lactate concentration were measured with a Stat Profile pHox Plus L analyzer (Model i-STAT 300, Abbott, America) at baseline.

Sublingual microcirculation was visualized with the aid of the CapiScope Handheld Video Capillaroscope (HVCS) system. Microcirculation is illuminated with a green light and the backscattered light is filtered, creating a high-contrast image of flowing red blood cell (RBC) in superficial vessels [16]. After removal of gross secretions with gauze, the camera was inserted in the mouth of the animal and positioned in the right sublingual fossa at a site approximately 4 cm proximal to the tip of the tongue. Determinations were made off-line by investigators blinded to the group assignment and hemodynamics.

Individual recordings of 10 seconds were analyzed off-line by a single individual using a score previously described by Spronk et al.[17], in which 0 represents “no flow,” 1 “markedly reduced flow,” 2 “reduced flow,” and 3 “normal flow.” We focused our analysis to small vessels (<20 μm), which are mostly capillaries, and calculated PVD and MFI of the sublingual microvasculature following established guidelines [18].

Vessel size was measured with a micrometer scale superimposed in the video display. Values were obtained at baseline, 1 min, and 5 min after start of chest compression, and 1 and 5 min after ROSC. To assess the degree of inter-observer reliability 50% of the recordings were randomly selected and evaluated by a second individual.

### 4. Statistical analysis

Statistical analysis was performed with SPSS software, Version 17.0 (SPSS Inc., Chicago, IL). Continuous variables were presented as mean ± SD when data were normally distributed or as a median (25th, 75th percentiles) when data were not normally distributed. Normal distribution of the data was confirmed with the Kolmogorov-Smirnov test. Variables were compared with parametric Student t test or Mann-Whitney U test for nonparametric data. Comparisons between time-based measurements within each group were performed with a paired sample Student t test. A two-sided p<0.05 was considered statistically significant.

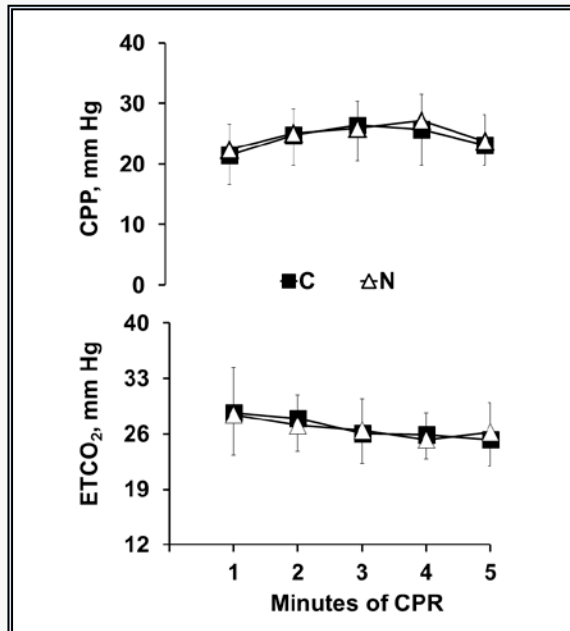
### Results

No baseline differences in body weight, heart rate, mean aortic pressure (MAP), right atrium pressure (RAP), ETCO<sub>2</sub>, PaO<sub>2</sub>, or arterial lactate were observed (Table 1).

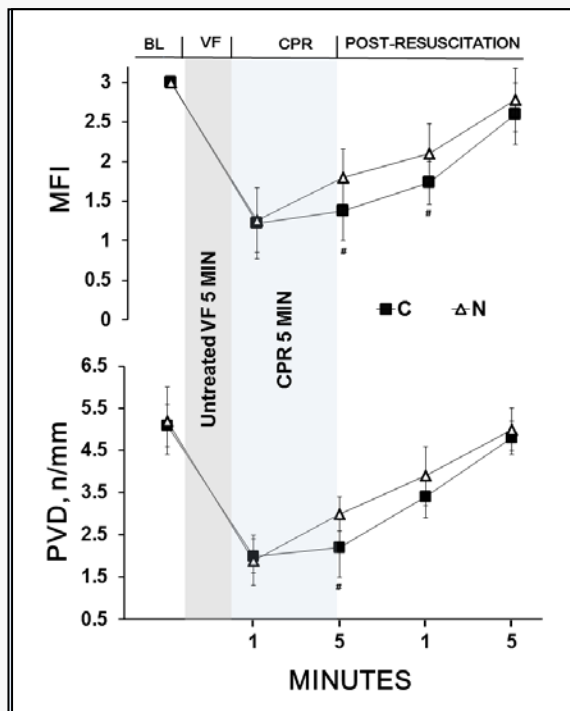
Coincident with the start of precordial compression, microcirculatory blood flow was partially restored in each animal. All animals were successfully resuscitated in both groups. There were no differences in the number of defibrillations and in the hemodynamics after 1 min and 5 min of precordial compression between two groups (Table 2).

There were no differences in CPP or ETCO<sub>2</sub> during the time of CPR between the two groups (Figure 1). Sublingual PVD and MFI were significantly improved in the NTG treated animals compared to those of the control animals during and after CPR (Figure 2).

Autopsy did not reveal any injury to the bony thorax, the thoracic, or the abdominal viscera.



**Figure 1:** Changes in hemodynamics during cardiopulmonary resuscitation. CPP, coronary perfusion pressure; ETCO<sub>2</sub>, end-tidal carbon dioxide; C group, control group; N group, administration of nitroglycerin at the onset of cardiopulmonary resuscitation.



**Figure 2:** Changes of sublingual microcirculation. BL, baseline; VF, ventricular fibrillation; CPR, cardiopulmonary resuscitation; MFI, microcirculatory flow index; PVD, perfused vessel density; C group, control group; N group, administration of nitroglycerin at the onset of CPR. #  $p < .05$  vs. the N group.

## Discussion

Sublingual PVD and MFI were greater in NTG treated animals than those of control animals during and after CPR. There were no differences in CPP or ETCO<sub>2</sub> between the two groups. All animals were successfully resuscitated in both groups.

Survival rates following sudden cardiac death remain dismal, in part due to our limited understanding of the pathophysiology of the extreme low-flow states of CA and CPR. Initiation of CPR and epinephrine administration until ROSC remains the treatment of choice in adults with VF at the time of CA [19]. While little controversy exists over the ability of epinephrine to increase rates of ROSC, there are no well-controlled trials of epinephrine to assess endpoints such as improved survival and neurologically intact survival. In theory, higher doses of epinephrine may increase coronary perfusion pressure, resulting in increased ROSC and survival from CA. However, the adverse effects of higher doses of epinephrine in the post-arrest period may negate potential advantages during the intra-arrest period. A number of trials have compared outcomes from standard-dose epinephrine with those of high-dose epinephrine. These trials did not demonstrate any benefit for high-dose epinephrine over standard-dose epinephrine for survival to discharge with a good Cerebral Performance Category score, survival to discharge, or survival to hospital admission [20-25]. In the CA setting, the overwhelming endogenous epinephrine release in association with exogenous epinephrine administration results in intense vasoconstriction and coronary vasoconstriction, thus further reducing the blood supply to the myocardium. During CPR before epinephrine was given, the plasma concentration of epinephrine in resuscitated patients was significantly lower than that in those that were not resuscitated, and

it was the same as after epinephrine was given [26].

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**Table 1:** Baseline characteristics

	C group	N group
Body weight, kg	41±1	39±1
Heart rate, beats/min	107±10	108±9
Mean aortic pressure, mmHg	109±8	113±10
Right atrium pressure, mmHg	3.3±0.5	3.4±0.7
ETCO <sub>2</sub> , mmHg	39.7±1.9	38.9±2.6
P <sub>i</sub> O <sub>2</sub> , mmHg	98±16	103±18
Arterial lactate, mmol/L	1.2±0.4	1.0±0.6

Values are presented as mean ± SD. C group, control group; N group, administration of nitroglycerin at the onset of cardiopulmonary resuscitation.

**Table 2:** Selected hemodynamics and outcomes of cardiopulmonary resuscitation

	C group	N group
<b>Hemodynamics at 1 min of CPR</b>		
Systolic AP, mmHg	63±22	66±27
Diastolic AP, mmHg	27±4	28±5
Systolic RAP, mmHg	37±10	34±9
Diastolic RAP, mmHg	5±2	7±3
<b>Hemodynamics at 5 min of CPR</b>		
Systolic AP, mmHg	62±17	59±14
Diastolic AP, mmHg	28±8	26±7
Systolic RAP, mmHg	40±13	35±9
Diastolic RAP, mmHg	7±3	8±1
Duration of cardiopulmonary resuscitation, mins	5(5-5)	5(5-5)
Numbers of defibrillation for ROSC	1(1-1)	1(1-1)

Values are presented as mean ±SD or median plus interquartile range. AP, aortic pressure; RAP, right atrial pressure; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; C group, control group; N group, administration of nitroglycerin at the onset of CPR.

The beneficial effects of NTG has been evaluated in multiple studies observing its effects in conjunction with epinephrine. Kitsou et al. compared efficacy of epinephrine and NTG combination to epinephrine alone and reported a significant increase in CPP during CPR in animals treated with the epinephrine-nitroglycerin combination. No significant difference was observed with regard to ROSC rates between the epinephrine treated animals and the animals treated with the drug combination [27]. Keith et al. declared

vasopressin plus nitroglycerin) significantly improved vital organ blood flow during CPR compared with epinephrine alone. Addition of nitroglycerin to the combination of low dose epinephrine with vasopressin during cardiac arrest may be beneficial [28]. Varvarousi et al. studied in the porcine model of asphyxial CA and found that the addition of nitroglycerin to vasopressin and epinephrine maintained elevated coronary perfusion pressure during asphyxia CA and resulted in significantly better

neurologic and histopathologic outcome in comparison with epinephrine alone [29].

Changes in macrocirculatory hemodynamics and gas exchange during CA and CPR, and especially CPP and ETCO<sub>2</sub>, have been investigated extensively as predictors of outcomes in the restoration of cardiac function [30,31]. Yet the “microcirculation,” and more specifically, the capillary exchange bed is likely to be the ultimate determinant of circulatory function. Low-dose NTG significantly reduces cardiac filling pressures and improves microvascular perfusion in patients admitted for acute heart failure [13]. NTG dose-dependently increases tissue perfusion in patients with severe heart failure, as observed by a decrease in central-peripheral temperature gradient and an increase in sublingual perfused capillary density [32].

CA results in global multi-organ ischaemic reperfusion (I/R) injury and is associated with significant morbidity and mortality [33]. I/R injury initiates its damage at a mitochondrial level and can be characterized by two stages. First, the ischaemic phase causes a decrease in oxygen delivery and malfunction of the respiratory chain complex, leading to anaerobic metabolism and inadequate adenosinetriphosphate(ATP) synthesis [34]. Consequently, all energy-dependent processes gradually cease their activity, leading to a sudden loss of the mitochondrial membrane potential and to mitochondrial calcium overload. Second, during the reperfusion phase, changes of the mitochondrial membrane potential leads to the opening of the mitochondrial permeability transition pore (mPTP) [35]. During reperfusion the sudden increase in oxygen delivery to the mitochondria and the opening of them PTP leads to a dramatic increase in reactive oxygen species production, which overwhelms the endogenous scavenging mechanisms [36]. The main sites of reactive oxygen species generation are mitochondrial complexes I and III. This sudden

increase in reactive oxygen species, along with calcium overloading, leads to the release of pro-apoptotic proteins, which initiates apoptotic signalling and cell death [37]. Moreover, the oxygen radicals increase endothelial injury, which causes dysfunction in the microcirculation, principally at the capillary level [38].

Post-resuscitation myocardial dysfunction is accompanied by reperfusion arrhythmias. The mechanism underlying the functional and electrophysiologic derangements in reperfusion arrhythmias is altered calcium homeostasis [39]. NTG has a protective role against arrhythmias that are induced by I/R injury. NTG through nitrogen oxide (NO) reduces incidence and duration of ventricular arrhythmias by blocking the calcium channel [40].

The limitations of our study are acknowledged below. First, PVD and MFI by use of an OPS were evaluated in a semi-quantitative way. Second, whether the beneficial effects of NTG on sublingual microcirculation could apply to other microvascular beds need to be investigated further. Third, whether the improvement of microcirculation by administration of NTG will reduce cellular dysfunction, organ failure, or mortality in animals with CA was not measured in the present study. Fourth, our study was performed on healthy juvenile pigs which were free of underlying disease and direct applicability to humans is not assumed. Fifth, because of gasping and chest compression during recording of microcirculation images, motion artifacts were observed.

## Conclusion

Using a porcine model of CA, we report that administration of NTG at the onset of CPR improves microcirculation without undesirable effects on macrocirculation during and after resuscitation. Whether the administration of NTG will reduce cellular dysfunction, organ failure, and mortality in

animals with CA should be investigated further. Also, dosing of NTG still needs to be optimized by monitoring vital tissue microcirculation.

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