Subcellular Injury of ASMC is Involved in the Genesis of Refractory Hypotension during Severe Shock

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Abstract

The Refractory Hypotension (RH) leads to severe hypoperfusion which results in vital organ failure and death. It has been shown that one of the main reasons for RH is Arteriolar Smooth Muscle Cell (ASMC) hyperpolarization, which results in the inhibition of L-type calcium channel and Ca2+ influx after vasoconstrictor stimulation that finally leads to refractory hypotension during severe shock. The activation of KATP channels by depletion of intracellular ATP content causes ASMC hyperpolarization. It is usually recognized that the depletion of ATP content originates from microcirculatory disturbance and refractory hypotension is only a functional problem of ASMC with treatment using vasopressors, and no morphological changes of smooth muscle cell were reported in RH. This review shows that mitochondrial damage is another important reason for depletion of ATP level and that protection of mitochondrial dysfunction can increase the blood pressure and survival rate during severe shock, which indicated that subcellular injury of ASMCs is involved in the genesis of refractory hypotension. Protecting and repairing ASMC subcellular injury is a new approach to treatment of severe shock.

Keywords: Refractory hypotension; Severe shock; Mitochondrial dysfunction; KATP channel; ASMCs hyperpolarization

Introduction

Refractory shock has been defined as the need for nor adrenaline infusion of >0.5 µg/kg/min despite adequate volume resuscitation. Mortality in these patients may be as high as 94% [1-3]. One of the reasons for death is refractory hypotension, which induces severe microcirculatory hypoperfusion with vital organ failure and death [4-6]. Therefore, it is important to investigate the exact mechanism of refractory hypotension and find a new approach to treatment of refractory hypotension during severe shock.

ASMC Hyperpolarization is the Basis for Pathogenesis of Refractory Hypotension during Severe Shock

An acute severe hemorrhage rat model was reproduced, in which the duration of experiment was maintained for 4 h (2 h for hemorrhage to MAP 30 mmHg and 2 h after reinfusion of the shed blood), then the ASMCs were isolated and the mitochondrial function was measured. The protectors against mitochondrial injury (Cyclosporine A (CsA), Resveratrol (Res), Polydation (PD)) were used to observe if protection from mitochondrial injury could improve refractory hypotension during severe shock. All experimental procedures were carried out in accordance with the U.S. National Institute of Health’s “Guide for the Care and Use of Laboratory Animals”, with the approval of Ethics Committee from Southern Medical University, Guangzhou, China.

The spinotrapezius muscle preparation was used for measuring vasoreactivity. The semi-quantitative arteriolar reactivity to Norepinephrine (NE) was tested by topical application of increasing concentration of NE until a threshold concentration of NE was reached, which resulted in complete cessation of blood flow in the transverse arteriole of spinotrapezius muscle for 10-20s [7]. It was shown that the NE threshold level in pre-bleeding shock group increased 15 times more than in the non-bleeding group [8,9]. Then the ASMC were isolated for measuring membrane potential and ion channels.

The resting membrane potential of ASMC changed from -36.7 ± 6.3 mv in sham group to -51.7 ± 9.1 mv in shock group, which showed existence of ASMC hyperpolarization. The ASMC hyperpolarization resulted in the inhibition of L-type calcium channel and Ca2+ influx
The Evidence for Mitochondrial Damage leading to ATP Depletion of ASMC during Severe Shock

Mitochondria are the power plant with 90% ATP production in cell. Mitochondrial damage may lead to energy exhaustion with ROS production, release of apoptosis enzymes, and calcium overload, which finally results in cell death. Therefore, mitochondrial damage is a subcellular injury, which indicates the disease course at an early stage of cell injury during severe shock. Mitochondrial swelling is a prominent phenomenon in ASMC during severe shock, in which the shape of mitochondria changes from normal sausage style with electron-dense matrix to spherical or irregular style and the cristae is destroyed and disappears with no electron-dense matrix during severe shock [28-31]. It is well known that the mitochondrial cristae are made of inner membrane, where the mitochondrial respiratory chain and ATP synthesis are located. Therefore, disruption of mitochondrial membrane and loss of defined cristae would result in depressed ATP production.

ASMC mitochondrial dysfunction can also been assessed by the opening of mitochondrial transition pore and reduction of mitochondrial membrane potential (Δψm), which can be measured by special fluorescent probe (Calcine-AM for measuring mPTP and JC1 for Δψm) under confocal microscope. It was demonstrated that the ASMC calcine value of Mean Fluorescence Intensity (MFI) in shock group was decreased by 68.8% of control group indicating substantial mPTP opening, and that the value of Δψm ASMCs was from 13.44 ± 7.73% in control group and increased to 80.34 ± 9.01% in shock group, indicating reduced mitochondrial membrane potential of ASMC during severe shock [28].

Intracellular ATP content of ASMC is an important index for assessing mitochondrial dysfunction. It was shown that the ATP level of ASMC in shock group decreased to 17.6 ± 7.9% of the control value. Treatment with Cyclosporine A (CsA), resveratrol (Res), and Polydatin (PD) increased ATP level to 32.7 ± 5.4%, 62.1 ± 1.5% and 90.7 ± 7.5% respectively [29]. The therapeutic effect of mitochondrial protectors (CyPD), Res, PD) provides counterevidence for mitochondrial dysfunction involved in the genesis of refractory hypotension [30].

Besides ASMC, mitochondria dysfunction also existed in diverse organs during severe shock, including brain neurons [31], small intestine epithelial cells [32], renal tubular epithelial cells [33,34], pulmonary arteriolar smooth muscle cells [35], hepatocytes [36], platelets [37], etc. Therefore, mitochondrial injury is a common pathway in severe shock. It will be very convenient to check platelet mitochondrial damage as an index of cell damage in severe shock, since taking blood samples is easy in clinics.

The pathogenesis factors of mitochondrial damage during severe shock include free radicals, calcium over load, cathepsin of lysosomes, etc. It was shown that with reduced SIRT1/3 activity, three kinds of mitochondria-related proteins (CyPD, SOD2, P53) would be over-acetylated, which led to mPTP opening, more ROS production and P53 transcription-independent apoptosis during severe shock, and that Polydatin (PD) might serve as an activator of SIRT1/3 [33-36].
mitochondria during severe shock have the following aspects: (1) inhibiting the opening of mitochondria permeability transition pore; (2) attenuating the production of reactive oxygen species; (3) modulating inner ion (Ca2+, K+) channels; (4) ameliorating energy substrate metabolism; (5) activating SIRT1/3 [29,30]. Three kinds of them were chosen for the experiment, i.e., Cyclosporine A (inhibiting mPTP opening), resveratrol (attenuating ROS production), and polydatin (activating SIRT1/3).

Administration of mitochondrial protectors (CaA, Res, PD) could partially recover the morphologic mitochondrial damage of ASMC, especially in the PD-treated group; the ATP content of ASMC reached 90.7 ± 7.5% of the control value, the MAP increased from 47.23 ± 11.20 mmHg in shock group to 89.38 ± 16.31 mmHg in PD-treated group 24 h after treatment, and the 24-h rat survival rate enhanced from 0/8 in shock group to 5/8 in PD-treated group [10]. Polydatin has therapeutic effect both on circulatory disturbance and on cellular injury during severe shock [38-43]. Polydatin has obtained the permission of CFDA (China Food and Drug Administration) and FDA (America) for clinical trials in China and America, respectively [9].

**Conclusion**

In summary, mitochondrial damage causes the depletion of ASMC ATP level and activation of KATP channels, which induces ASMC hyperpolarization with refractory hypotension during severe shock. On the other hand, refractory hypotension after anti-shock therapy is also a clinical sign for diagnosis of subcellular injury during severe shock, since refractory hypotension implies a morphologic injury of arteriolar smooth muscle cells. Protecting and repairing mitochondrial dysfunction is a new way to treatment of severe shock.

**Ethics Approval and Consent to Participate**

All experimental procedures were carried out in accordance with the U.S. National Institute of Health’s “Guide for the Care and Use of Laboratory Animals”, with the approval of Ethics Committee from Southern Medical University, Guangzhou, China.

**Consent for Publication and Availability of Supporting Data**

Agree to conditions of submission, BioMed Central’s copyright and license agreement and article-processing charge (APC).

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