Austin Journal of Pharmacology and Therapeutics

Open Access

Austin Publishing Group

Review Article

Subcellular Injury of ASMEs Involves in the Genesis of Refractory Hypotension in Severe Shock

Zhao K*

Department of Pathophysiology, Southern Medical University, China

***Corresponding author:** Ke-seng Zhao, Department of Pathophysiology, Southern Medical University, Guangzhou 510515, China

Received: March 05, 2021; **Accepted:** April 02, 2021; **Published:** April 09, 2021

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 Ca^{2+} influx after vasoconstrictor stimulation, that finally leads to refractory hypotension during severe shock. The activation of K_{ATP} 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; K_{ATP} channel; ASMCs hyperpolarization

Introduction

Refractory shock has been defined as the need for noradrenaline 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 the key lab for shock and microcirculation research, 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.

The spinotrapezius muscle preparation of rat 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 10s to 20s [7]. It was shown that the NE threshold level in 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 mv ± 6.3 mv in sham group to -51.7 mv ± 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 Ca^{2+} influx after vasoconstrictor stimulation, which finally led to refractory hypotension during severe shock. Administration of mitochondrial protectors could partially restore ASMC hyperpolarization [9-15].

The Activation of K_{ATP} Channel Leads to ASMC Hyperpolarization during Severe Shock

ATP-sensitive potassium channels (K_{ATP}) are physiologically activated by decreasing the cellular ATP concentration and by increasing the cellular concentrations of hydrogen ion and lactate, which is a mechanism linking cellular metabolism with vascular tone and blood flows. It was demonstrated that the K_{ATP} channels in ASMC are activated following severe hemorrhagic shock, which showed that more K_{ATP} channels in recorded patches are in activity with the increased conductance and mean open probability; it was also shown that the cause of K_{ATP} activation in ASMCs during severe shock is ATP depletion and intracellular acidosis, which showed that ASMC ATP level reduced to 17.9% ± 6.4% of control level and intracellular pH value (pHi) changed from 7.08 ± 0.8 of the control level to 6.63 ± 0.13 in rats during severe hemorrhagic shock [16-18]. In the meantime, ASMC hyperpolarization was also recorded, which might be produced by the activation of K_{ATP} channels, since inhibition of the K_{ATP} channels with glybenclamide could reverse

Citation: Zhao K. Subcellular Injury of ASMEs Involves in the Genesis of Refractory Hypotension in Severe Shock. Austin J Pharmacol Ther. 2021; 9(2).1131.

the hyperpolarization. Based on the study of mitochondrial function during severe shock, a new approach to treatment of severe shock was put forward, i.e., administration of restituting vasoreactivity agent (glybenclamide) first, followed by giving vasopressor (NE or dopamine) and transfusion of blood [19-23].

The Reason for the Depletion of ATP Content in ASMC during Severe Shock

The deficiency in nutrients and oxygen caused by failure of circulation may lead to low ATP production and improvement of microcirculation is the principal treatment in severe shock. It was, however, reported that the ATP depletion which occurred during shock remained as long as 48 h after treatment with restitution of the blood pressure [24]. It was shown that the depressed ASMC ATP level accompanied by the activation of K_{ATP} channels [10]. These indicated that depletion of ASMC ATP level might not only result from deficiency of ATP raw material, but also from the damage of ATP-producing factory. This is an important event, since the refractory hypotension is usually recognized to be only a functional problem of ASMC, so that the treatment of severe shock normally is to apply more and more vasopressors to the patients, even if it is usually failed to enhance the blood pressure, because the vasopressors cannot improve the morphology damage in smooth muscle cell.

Doctors do not pay attention to the treatment of ASMC injury in refractory hypotension, because they do not know that the shock patient might enter into cell injury stage which could have morphology damage before ultimately organ failure occurs. When subcellular mitochondrial damage occurs during refractory hypotension leading to ATP depletion *via* cytopathic hypoxia, the principle of pharmaceutical therapy should include protecting and repairing ASMC injury during severe shock [21-27].

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 disappeared on mitochondria which can be observed under electro-microscope with electron-lucent 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 [26,27].

ASMC mitochondrial dysfunction can also been assessed by the opening of mitochondrial transition pore and reduction of mitochondrial membrane potential ($\Delta\psi m$), which can be measured by special fluorescent probe (Calcein-AM for measuring mPTP and JC1 for $\Delta\psi m$) under confocal microscope. It was demonstrated that the ASMC calcein 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 $\Delta\psi m$ 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 accessing 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 (CsA, Res, PD) provides a counterevidence for mitochondrial dysfunction involved in the genesis of refractory hypotension (see next).

Besides ASMC, mitochondria dysfunction also existed in diverse organs of in severe shock, including brain neurons [31], small intestine epithelial [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 much convenient to check platelet mitochondrial damage as a marker to reflect the subcellular damage in severe shock, as to take a blood sample will be much easier for clinic patient.

The pathogenesis factors of mitochondrial damage during severe shock include free radicals, calcium over load, casepsin 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].

Protecting and Repairing Mitochondrial Dysfunction is a New Approach to Treatment of Severe Shock

According to the pharmacologic effect, drugs targeting 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 (Ca^{2+}, K^{+}) channels; (4) ameliorating energy substrate metabolism; (5) activating SIRI1/3[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 (CsA, 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 mmHg ± 11.20 mmHg in shock group to 89.38 mmHg ± 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 the therapeutic effect both on circulatory disorder 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 K_{ATP} 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 dysfunction is a new way to treatment of severe shock.

Funding

This work was supported by the National Science Foundation of China (No. 3880933, No. 39270852, No. 39830400, No. 30070735, No. 30672179), Chinese National Programs for High Technology Research and Development (Z18-02), and National Key Science and Technology Project (2011ZX09101-002-08).

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