

Review

Hemorheologic events in severe shock¹

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Abstract. Persistent low perfusion and low blood pressure are the two major events in the pathogenesis of irreversible shock. This review is focused on our recent study on the mechanism of, and a new therapeutic approach to the two events in IS. One of the main causes of persistent low perfusion are leukocyte adhesion on venule walls and plugging in capillaries which comes from the low wall shear stress or shear rate, and high leukocyte–endothelial adhesion force in IS. However, blockade of leukocyte adhesion by monoclonal antibodies against the adhesion molecules can only attenuate the number of sticking WBC in venules, but cannot make an appreciable improvement in capillary reflow and survival rate in IS, because it is difficult for the agents to flow into an obstructed capillary. We have shown that the administration of Polydatin, a crystalline product isolated from a traditional Chinese medicine, can restore the pulse pressure with high survival rate in irreversible shock. With an increase in pulse pressure, and the highly dispersive force resulting from pulsatile blood flow, the stationary blood cells can be pushed away from the obstructed capillary and thus promote capillary reflow. Therefore, enhancement of pulse pressure is a key factor for the treatment of low perfusion in irreversible shock. Hyperpolarization of arteriolar smooth muscle cells occurs in irreversible shock, which inhibits the potential-operated calcium channel and the influx of Ca^{2+} in arteriolar smooth muscle cells stimulated by norepinephrine, and finally leads to low vascular contractile responsiveness with refractory hypotension in irreversible shock. Activation of the potassium channels K_{ATP} and BK_{Ca} is involved in arteriolar smooth muscle cells hyperpolarization. In irreversible shock, ATP depletion, intracellular acidosis, $ONOO^-$ formation, and enhancement of a calcium spark results in activation of K_{ATP} and BK_{Ca} and consequent arteriolar smooth muscle cell hyperpolarization. Therefore, a new therapeutic strategy for refractory hypotension was suggested, including blockade of potassium channel activation to reconstitute vasoreactivity and the administration of vasopressors to elevate blood pressure in the treatment of irreversible shock.

Keywords: Leukocyte plugging, capillary no-reflow, low vasoreactivity, refractory hypotension, shock

1. Introduction

Severe or irreversible shock (IS) is the final stage of shock, which is a life threatening situation with the therapeutic measures being ineffective. It is believed that IS can be reversed, if the pathogenesis of IS is well understood [6]. In order to study the pathogenesis of IS and to look for a new approach to treat severe shock, a severe hemorrhagic shock model was reproduced in the rat [41,44]. First, the mean arterial pressure (MAP) was lowered from 100 to 40 mmHg by bleeding, the hypotension lasting for 1 hr before the shed blood was reinfused. After reinfusion two different trends could be seen: immediately upon volume restoration, MAP was restored to 100 mmHg, but gradually decreased within 120 min in

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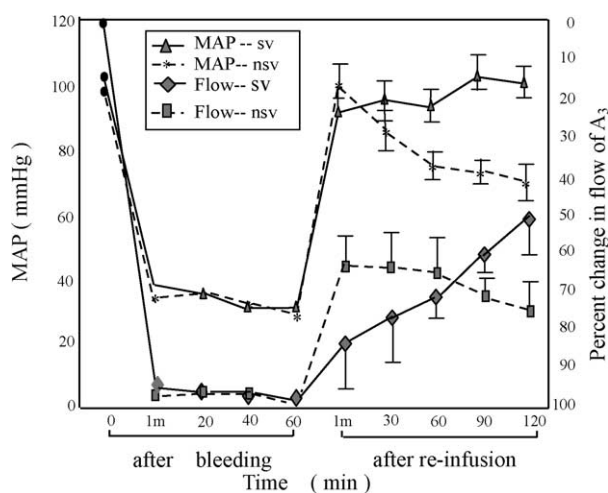


Fig. 1. A dissociation phenomenon of recovery between the macrocirculation (mean arterial pressure, MAP) and microcirculation (changes in blood flow) after treatment in IS [42]; sv: survival group, solid lines; nsv: non-survival group, dashed lines.

the non-survival group, while it remained normal in the survival group. On the other hand, the volumetric blood flow in A_2 arterioles of the cremaster muscle tended to decrease throughout reaching 25.6% of the control level at the end of 2 h after reinfusion in the nonsurvival group, but tended to increase gradually and reach to 48.2% of control value in the survival group. The experiment indicates that a dissociation phenomenon of recovery between the microcirculation blood pressure (BP) and blood flow after treatment is applied in severe shock [42] as illustrated in Fig. 1. That implies that persistent low perfusion and low blood pressure are the two major hemorheologic events involved in the pathogenesis of IS. It is important to find the mechanism and treatment of no-reflow and refractory hypotension in IS, since recovery of the microcirculation is closely related to the therapeutic effect and survival rate in severe shock [9,31]. This review is focused on the 2 hemorheologic events based on our research work.

2. Leukocyte adhesion and low perfusion in IS

Based on the data shown in Fig. 1, high resistance to blood flow existed after treatment of severe shock, since in the survival group, the volumetric blood flow in the arteriole was only half that of the control 2 h after treatment, although the blood pressure had returned to nearly normal. Such a high flow resistance resulted from leukocyte adhesion and plugging in microvessels, as no apparent vasoconstriction was seen after treatment in the survival group.

2.1. Leukocyte activation

Leukocyte activation was determined using the nitroblue tetrazolium test (NBT). A burn shock model was reproduced in the rat by scalding the lower trunk and lower extremities with 80°C water for 30 seconds (covering 35–40% of total body surface area). The NBT positive fraction of rat activation increased from $2.8 \pm 2.6\%$ of the pre-burn level to $7.7 \pm 2.0\%$ and $10.9 \pm 3.4\%$ at 2 h and 5 h post-burn, respectively. The NBT % leukocyte activation was negatively related to the survival time of burned animals (Fig. 2). If the NBT positive % activation remained unchanged within the 5 h postburn period or first

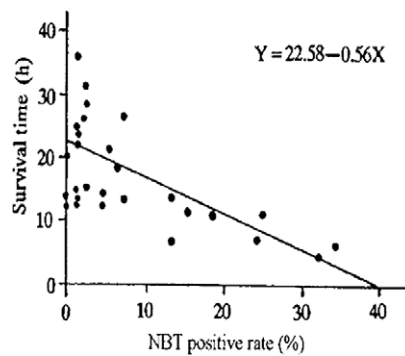


Fig. 2. The relationship between nitrotetrazolium test positive rate at 5 h post-burn and the survival rate in a rat with burn shock [30].

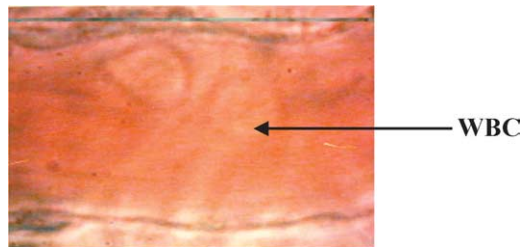


Fig. 3. Two leukocytes adhere to a venule wall in hemorrhagic shock.

increased to a high level and then decreased to normal levels, the survival time was much longer than that in the animal with continuously increasing NBT % activation [30]. These experiments indicated that leukocyte activation is involved in the pathogenesis of severe shock, and that the NBT could serve as an indicator of the irreversibility in shock [2,30].

2.2. Leukocyte adhesion

The number of leukocytes adhering to the venule wall in shock can be counted under a microscope (Fig. 3). Thirty min post hemorrhage, the number of adhering leukocytes on the venule wall (V_3) of spinotrapezius muscles increased by 16–20 fold over that in the control. Leukocyte adhesion to venules leads to an increase in vessel resistance, the inverse of vessel conductance. Thirty min post-bleeding, the diameter of the V_3 venule decreased to 87.5% of the control value, but the vessel conductance decreased only to 23.2% of the control, as the number of adhesive WBCs increased 20 times [40]. Using the method of stepwise multiple logistic regression, it was shown that the change in vessel conductance was closely related to that of the number of adhering WBC number and the vessel diameter, but that the change in adhesive WBC number was much greater than that of diameter. The correlation coefficient between the number of adhesive WBC and vessel conductance was -0.94 (Fig. 4). With increased flow resistance and decreased driving pressure, blood flow in the V_3 venule was only 8.9% of the control value 30 min post bleeding, which indicated that leukocyte adhesion is involved in low perfusion in IS.

Leukocyte adhesion to venular walls is closely related to 2 factors: one is the wall shear stress or wall shear rate, which prevents leukocyte adhesion; the other is the leukocyte–endothelium adhesive force, which comes from interactions between adhesion molecules that promotes leukocyte adhesion. In

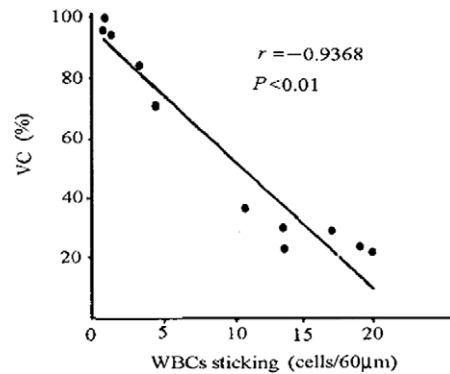


Fig. 4. The relationship between vessel conductance (VC) and the number of adhesive leukocytes in venules during hemorrhagic shock [40].

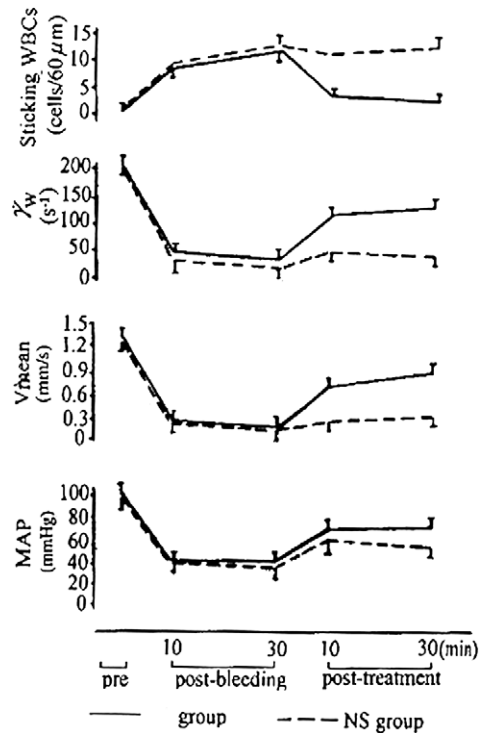


Fig. 5. The alteration of sticking leukocytes (WBC), wall shear rate (γ_w), blood velocity (V_{mean}), and mean arterial pressure (MAP) in hemorrhagic shock [40]. PD group: Polydatin-treated group; NS group: normal saline-treated group.

hemorrhagic shock it was found that the number of adhering leukocytes was negatively related to the mean blood velocity and wall shear rate indicating that the large number of adhering leukocytes could be partially reduced with the recovery of blood pressure, blood velocity, and wall shear rate after treatment in hemorrhagic shock (Fig. 5) [40]. It was shown that in rabbits with endotoxic shock, produced by injection of LPS (50 $\mu\text{g}/\text{kg}$, *E. coli* O111:B4, Sigma), the expression of adhesion molecules (LFA-1, Mac-1) on leukocytes increased and the administration of LFA-1 monoclonal antibody could partially reduce the number of adhering WBCs (Table 1) [32].

Table 1
Number of WBCs sticking on venule wall in endotoxic shock (number/150 μ m length of venule)

Group	Pre-injection	Post-injection of LPS		
		0.5 h	2 h	3 h
LPS	0.9 \pm 0.3	4.9 \pm 1.2	8.7 \pm 1.2	10.9 \pm 1.4
LPS + LFA-1 mab-treated	1.1 \pm 0.5	1.9 \pm 0.7	2.0 \pm 0.2*	2.9 \pm 0.7*
LPS + NS-treated	0.7 \pm 0.3	8.7 \pm 4.9	11.0 \pm 4.6	14.0 \pm 4.3

* $p < 0.01$ vs LPS or LPS + NS-treated group.

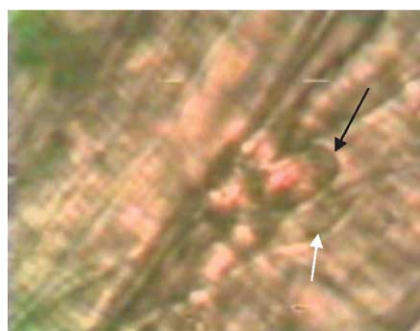


Fig. 6. Leukocyte plugging in capillary of spinotrapezius muscles in rats with hemorrhagic shock. White arrow shows that the endothelial nucleus protrudes to the lumen, leading to capillary narrowing. Black arrow shows an elongated-deformed leukocyte plugging flow in the capillary.

2.3. Leukocyte plugging

At any given time under normal conditions, a finite number of capillaries (20–25%) are seen to contain one or more leukocytes in transit [44]. Flow in these capillaries is slowed down appreciably. With bleeding and the accompanying hypotension, active flow is interrupted due to the presence of an elongated, deformed leukocyte that is trapped in the narrow capillary. The endothelial nucleus of the capillary usually protrudes into the lumen and leads to capillary narrowing, where leukocytes obstruct flow in the capillary in shock (Fig. 6). Although the incidence of leukocyte plugging is difficult to quantify under hypotension, the presence of leukocyte plugs becomes strikingly apparent as the blood pressure is elevated by blood replacement [44]. It was shown using a carbon reperfusion technique [2] that all arterioles and venules could be reperfused and only the capillaries in diverse organs developed no-reflow after treatment of irreversible hemorrhagic shock. It was also demonstrated that leukocyte plugging was the key factor in the no-reflow phenomenon and restoration of blood pressure was unable to remove such trapped leukocytes [3]. Capillary no-reflow post treatment of shock was first observed in animal models, but it was recently confirmed in a patient with myocardial infarction, which indicated that no-reflow was an independent predictor of death [1,21]. Therefore, it becomes important to look for an approach to treat WBC plugging with no-reflow in irreversible shock [3,23].

Blockade of leukocyte adhesion with different agents (e.g. SOD, allopurinol, monoclonal antibody of LFA₁, ICAM-1, TNF α , etc.) can attenuate the number of sticking WBC in venules during shock, but cannot make an apparent improvement in capillary reflow and prolong the survival time in shock, since it is difficult for these agents to arrive in an obstructed capillary [5,32,34]. In order to obtain capillary reflow, therefore, it is important to apply force to abolish trapped leukocytes in the capillary.

2.4. Pulse pressure and dislodging of trapped WBC

Polydatin (PD) is a monocrystalline product isolated from a traditional Chinese herb medicine – *Polygonum cuspidatum*. Administration of PD increases both survival time and survival rate. It was shown that the average survival time in the PD-treated group was 3-fold greater than that in the NS-treated group with half of them surviving more than 24 h in rat with burn shock (Table 2), and 5-fold greater than that in the NS-treated group with 8/10 of the rabbits surviving more than 48 h with hemorrhagic shock (Table 3) [35,36,45].

It has been reported that functional capillary density (FCD) is the primary determinant of survival in shock [7,28]. So it is reasonable to deduce that Polydatin is able to bring about capillary reflow by increasing FCD, since PD can significantly increase the survival rate in severe shock. We use Polydatin as a tool to see how it can promote capillary reflow in shock. A 2-camera simultaneously recording system was used in the experiment: one for recording the blood pressure curve on the screen of a dynograph, another for recording flow in the microcirculation under a microscope (Fig. 7). Administration of PD led to an increase of pulse pressure (PP), and with the widening of pulse pressure, a highly dispersed

Table 2
Effect of Polydatin (PD) on survival rate of rat with burn shock

Group	Average survival time (h)	Survival rate (24 h)
Simple burn	4.3 ± 0.8	1/10
Burn + NS-treated	6.6 ± 0.6	0/10
Burn + PD-treated	19.3 ± 3.0*	6/12*

* $p < 0.01$ vs simple burn group and NS-treated group.

Table 3
Effect of Polydatin (PD) on survival rate of rabbit in hemorrhagic shock

Group	Body weight (kg)	Blood loss (%)	Average survival time (h)	Survival rate		
				24 h	48 h	72 h
NS	2.07 ± 0.41	41.4 ± 3.7	15.4 ± 6.4	1/10	0	0
PD	2.03 ± 0.26	44.4 ± 5.6	84.6 ± 19.9*	9/10*	8/10*	6/10*

* $p < 0.01$ vs NS-treated group.

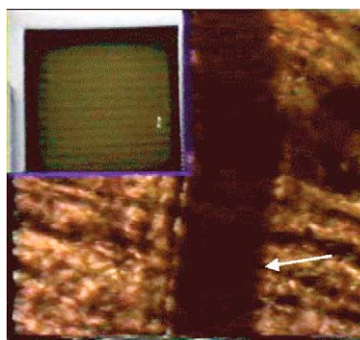


Fig. 7. A picture taken by a 2-camera simultaneously recording system: one for recording BP curve (upper left), the other for the microcirculation. Arrow indicates an entrance of a capillary, in which a pulsatile blood movement occurred to push the stationary blood cells.

force that resulted from the pulsatile motion of the blood at the entry of the capillaries. The force was able to push the stationary blood cells away from the obstructed capillary and bring about reflow in the capillary. The effect of Polydatin on PP at 1 h post hemorrhage is given in Table 4, which shows that PP had decreased to half the pre-bleeding value. However, at 10 min after treatment the PP value in the PD group had increased from the pre-bleeding value with all animals having open capillaries.

The reduced cardiac output (CO), stroke volume index (SVI), and $\pm dp/dt$ max of in the left ventricle increased immediately after injection of PD in hemorrhagic or burn shock in the rat (Fig. 8). PD treatment also led to a significant increase arteriolar diameter (Table 5). It was also shown that administration of Polydatin increased $[Ca^{2+}]_i$ in a single myocardial cell leading to an enhancement of contractility, activation of the ATP-sensitive potassium channel (K_{ATP}) in arteriolar smooth muscle cells (ASMC), and a decreased pH_i value in ASMC with a corresponding decrease in ASMC $[Ca^{2+}]_i$. The activation of the K_{ATP} channel leads to hyperpolarization of ASMC, which inhibits the potential operated calcium channel (POC) with a decrease in ASMC $[Ca^{2+}]_i$. The reduction of ASMC $[Ca^{2+}]_i$ and pH_i results in vasodilatation and decreased total peripheral resistance (TPR). The increase of $[Ca^{2+}]_i$ in myocardial cells enhances heart function with increased systolic blood pressure (SBP) [37]. However, PD only

Table 4
The effect of Polydatin (PD) on PP and capillary reflow in rat with hemorrhagic shock

	Prebleeding		1 h post bleeding		10 min post-treatment	
	NS	PD	NS	PD	NS	PD
MAP (kPa)	16.05 ± 0.28	17.44 ± 0.49	5.29 ± 0.05	5.28 ± 0.05	7.43 ± 0.37	8.62 ± 0.45
PP (kPa)	2.51 ± 0.36	2.46 ± 0.01	1.39 ± 0.15	1.21 ± 0.19	1.97 ± 0.18	3.08* ± 0.37
Case of open capillary	12/12	16/16	1/12	3/16	2/12	16/16**

Note: The medicine was intravenously given 1 h after hemorrhagic shock.

* $p < 0.05$, ** $p < 0.01$ vs NS-treated group.

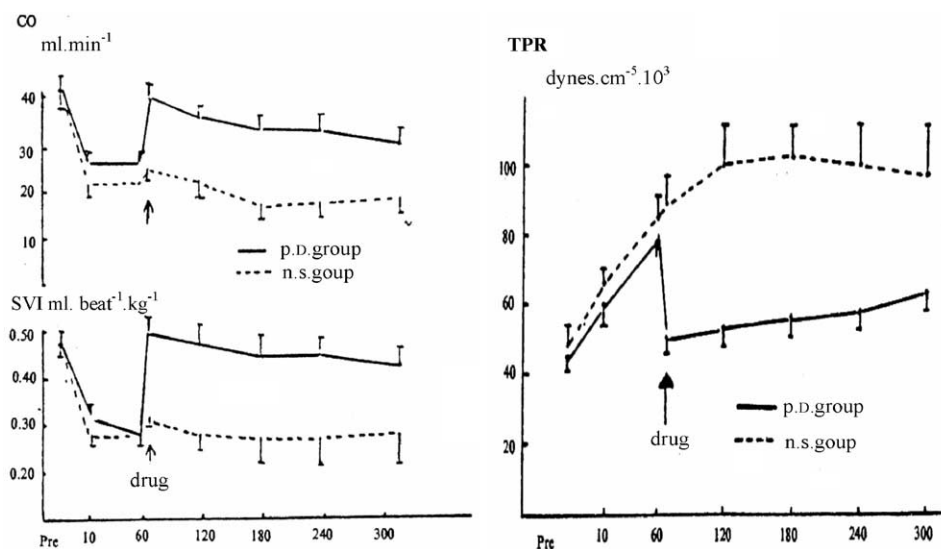


Fig. 8. Effect of Polydatin on cardiac output (CO), stroke volume index (SVI) and total peripheral resistance (TPR) in a rat with burn shock [35].

Table 5
Effect of PD on diameter of microvessels in cremaster muscle

Group	Pre-application (μm)	Post-application (μm)
A ₂ -arteriole	77.3 \pm 6.0	83.7 \pm 5.9*
A ₃ -arteriole	42.9 \pm 4.2	49.2 \pm 5.0*
V ₃ -venule	56.2 \pm 3.7	58.0 \pm 5.1

* $p < 0.05$ vs pre-application.

has a moderate effect on the elevation of SBP (from 40 to 65 mmHg), since it also dilates arterioles and reduces TPR in the treatment of shock. Therefore, both effects of PD on heart function and the microcirculation leads to the recovery and enhancement of pulse pressure with a highly dispersed force coming from the pulsatile blood flow, a key factor in promoting the capillary reflow and in coordinating the macrocirculation and microcirculation in the treatment of severe shock.

3. Low vasoreactivity and refractory hypotension in IS

Vasoreactivity is significantly reduced in severe shock, which leads to refractory hypotension with death after anti-shock treatment. It has been reported that metabolite accumulation, energy exhaustion, desensitization of adrenergic receptors, and the effect of cytokines (NO, ET, etc.) were thought to contribute to vascular hyporeactivity in IS [12,15,26,27]. However, the final common pathway to low vasoreactivity in severe shock is via reduced responsiveness of arteriolar smooth muscle cells to contraction by vasoconstrictors. What happens to ASMC in the reduced contraction state is still unknown. Our research work is focused on the change in the basic mechanism of ASMC contraction in IS. Our protocols include the following: reproduce a shock model in the rat; preparation of a microcirculation for observation under a microscope, and detection of its vasoreactivity to NE; isolate ASMCs using the pronase E enzymatic digestion method in order to measure what happens to ASMC by means of patch clamp and fluorescent probe techniques, and to observe if the low vasoreactivity and low blood pressure can be improved after the alterations in ASMC are restored.

3.1. Hyperpolarization of ASMC

Vasoreactivity is associated with the excitability and contractility of ASMC. Changes of membrane potential and intracellular calcium concentration are the major factors affecting smooth muscle excitation and contraction [16,17]. However, until recently, few studies have been conducted to determine the role of vascular membrane potential in the development of vascular hyporeactivity, and there are few reports in the literature regarding membrane potential and calcium kinetics in ASMC following shock [4, 13,14,24]. It was shown by us that the resting potential of ASMC increased from a pre-bleeding value of -36.7 ± 6.3 mV to -51.0 ± 9.1 mV at 2 h post shock, indicating the appearance of ASMC hyperpolarization in severe shock. Meanwhile the NE threshold concentration for an A₃ arteriole increased 15 times from the prebleeding value (from 0.17 ± 0.03 to 2.50 ± 0.65 $\mu\text{g/ml}$), which meant the appearance of low vasoreactivity in severe hemorrhagic shock (Table 6). ASMC hyperpolarization led to inhibition of the potential operated calcium channel (POC), resulting in a reduction of the $[\text{Ca}^{2+}]_i$ of ASMC stimulated by NE, which originally should be markedly increased in severe shock. Thus, the level of increased $[\text{Ca}^{2+}]_i$ in ASMC following NE stimulation in the shock group was only 50.1% of the value in the control group, which can finally lead to a decreased vessel contraction [38].

Table 6
ASMCs hyperpolarization with increased NE threshold in hemorrhagic shock

	Prebleeding	20 min post shock	2 h post shock
ASMCs resting potential (mV)	-36.7 ± 6.3	$-29.2 \pm 5.3^*$	$-51.0 \pm 9.1^*$
NE threshold value ($\mu\text{g/ml}$)	0.17 ± 0.03	$0.05 \pm 0.02^*$	$2.5 \pm 0.68^*$

* $p < 0.01$ vs prebleeding value.

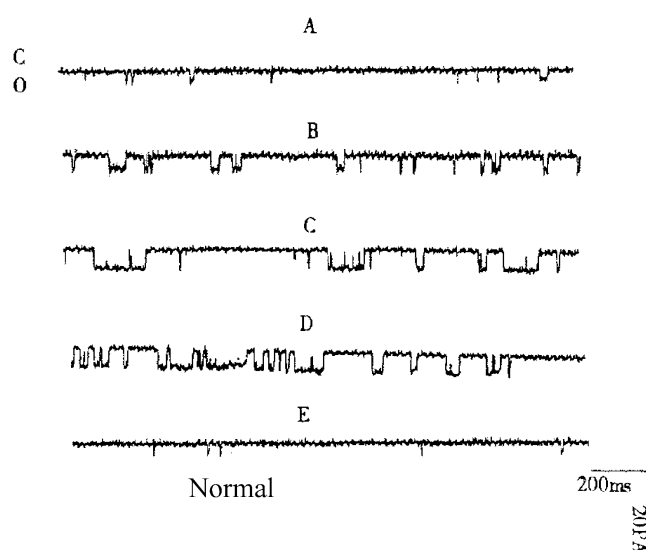


Fig. 9. Single ATP-sensitive K^+ channel currents of ASMC from a normal rat [10]. Holding potentials are A: -20 mV, B: -40 mV; C: -60 mV from cell-attached patch; D: -60 mV from inside out patch; and E: 3.5 mmol/l ATP was applied intracellularly and the channel activities were almost completely blocked.

3.2. Activation of K^+ channel

Potassium channels represent the dominant ion conductance of vascular smooth muscle membranes, play a major role in the regulation of membrane potential, and participate in the mechanism of vasoconstriction on the arteriolar wall [8,33]. Therefore, the condition of the ASMC K^+ channel needs to be explored in order to investigate the pathogenesis of ASMC hyperpolarization in IS. It was shown that the ATP-sensitive potassium channel (K_{ATP}) in ASMC was activated in severe shock, in which the open probability (P_o) of K_{ATP} in the shock group increased by 12.5 fold over that in the control group [10] (Figs 9 and 10). It was demonstrated that intracellular acidosis of ASMC, in which the pH_i level decreased from 7.08 ± 0.18 in the controls to 6.63 ± 0.11 in shock, can activate the K_{ATP} channel and lead to low vasoreactivity in IS. Treatment with ATP channel blocker (glybenclamide) and alkaline solution (NaHCO_3) improved the vasoreactivity of animals in shock [11].

In severe hemorrhagic shock, it was shown that the large conductance calcium-activated potassium channel (BK_{Ca}) of ASMC was also activated with an increased single BK_{Ca} channel open probability. The calcium spark is usually released by the sarcoplasmic reticulum and can activate a cluster of BK_{Ca} channels in the cell membrane, leading to the appearance of a spontaneous transient outward current (STOC) in ASMC. It was demonstrated that the calcium spark and the STOC in ASMC were enhanced

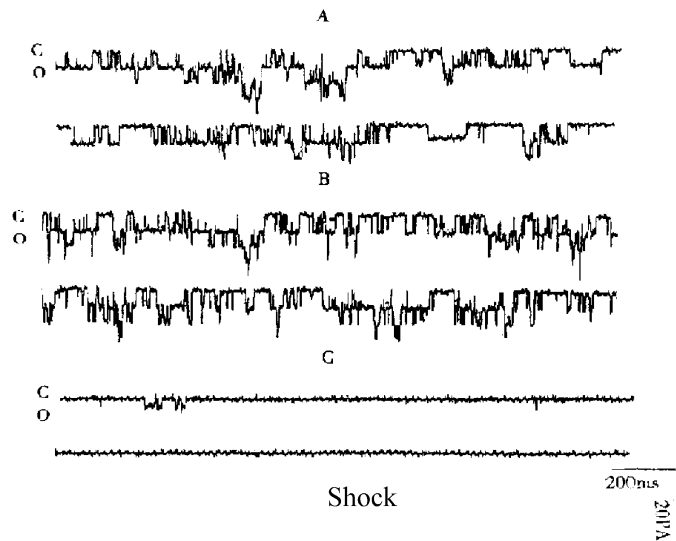


Fig. 10. Single K_{ATP} channel currents of ASMC from shock rats [10]. Holding potentials -60 mV A: recorded in cell-attached patch; B: recorded in inside-out patch; C: 0.5 mmol/l ATP was applied; open and closed states are indicated by O and C.

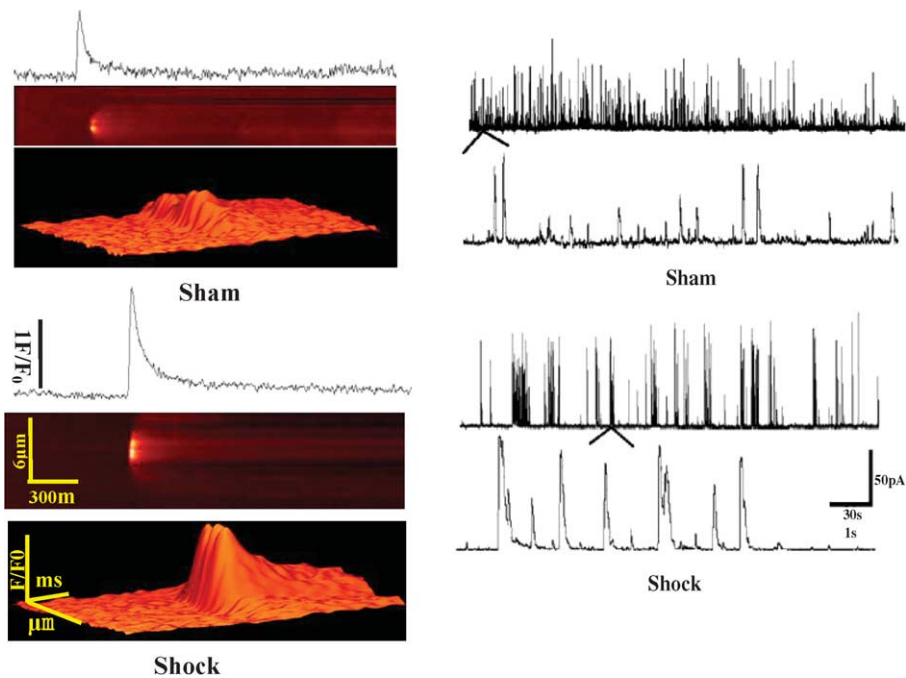


Fig. 11. Calcium spark (left side) and STOC (right side) of ASMC enhanced in shock.

in IS (Fig. 11). It was also shown that the hyperpolarization of ASMC was not caused by NO itself, but by $ONOO^-$ ($NO + O_2^-$), which could activate the BK_{Ca} channels with the increase of STOC frequency and amplitude [18–20,39] (Fig. 12). Treatment with BK_{Ca} blocker (ChTX) and oxygen free radical scavenger (tiron) improved the vasoreactivity in shock animals.

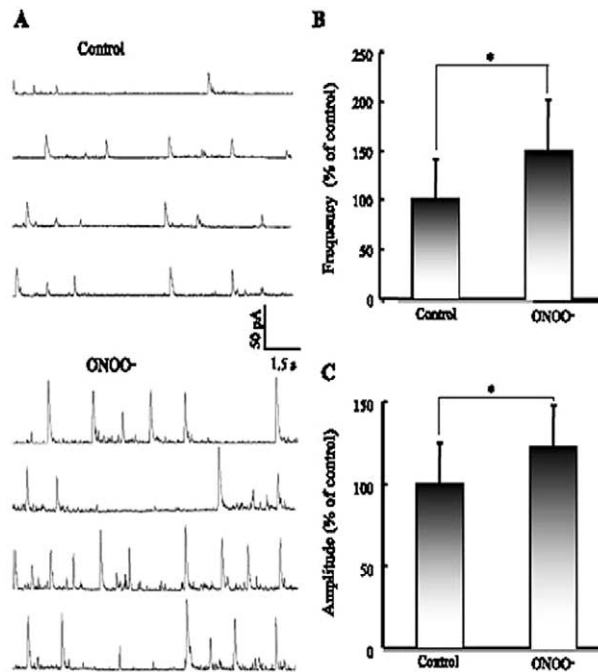


Fig. 12. Effect of authentic ONOO^- on STOC in mesenteric ASMCs [20]. A: STOCs recorded in ASMC under control conditions and after application of $10 \mu\text{M}$ ONOO^- . B: Summary results showing change in normalized STOC frequency after ONOO^- application. C: Changes in normalized STOC amplitude after ONOO^- treatment; * $p < 0.05$ vs control ($n = 4$).

3.3. Restitution of vasoreactivity

According to the work mentioned above, a scheme for a low vasoreactivity mechanism in severe shock was figured out, as shown in Fig. 13. The scheme indicates that the main factors for vascular hyporeactivity in IS are ASMC hyperpolarization and K^+ channel activation. Thus, blockade of K^+ channel activation might restore the membrane potential and vasoreactivity.

It was shown in isolated ASMC that glybenclamide (GL), a selective blocker of the K_{ATP} channel, might decrease the level of ASMC hyperpolarization by increasing NE-stimulated $[\text{Ca}^{2+}]_i$ in the late stage of severe shock. Also, tiron (TI), a scavenger of oxygen free radicals, might decrease the formation of peroxynitrite (ONOO^-) with restoration of ASMC membrane potential. It was demonstrated that glybenclamide and tiron can serve as vasoreactivity-restituting agents in the treatment of severe hemorrhagic shock in the rat. The protocol includes bleeding, maintaining MAP at 40–44 mmHg for 2 h, and treating with restituting agents (GL + TI) or a control solution (NS, DMSO), followed by injection of dopamine and re-infusion of the shed blood. It was demonstrated that 2 h after hemorrhage, the value of the NE threshold concentration was 15 times greater than the concentration pre-hemorrhage. Moreover, 2 h after treatment the NE threshold value decreased significantly in the restituting agent (RA) group, in striking contrast to the continuous enhancement of the NE threshold value in the control group. With the recovery of vasoreactivity, the effect of dopamine on the elevation of MAP in the RA group was 1.8 and 1.9 times as much as that in the NS or DMSO group. A trend of steadily increasing MAP post-treatment appeared in RA group in which the value of MAP in each time point was much higher than that in NS group (Fig. 14). Finally, the survival rate in RA group was much higher than that in control group [43]

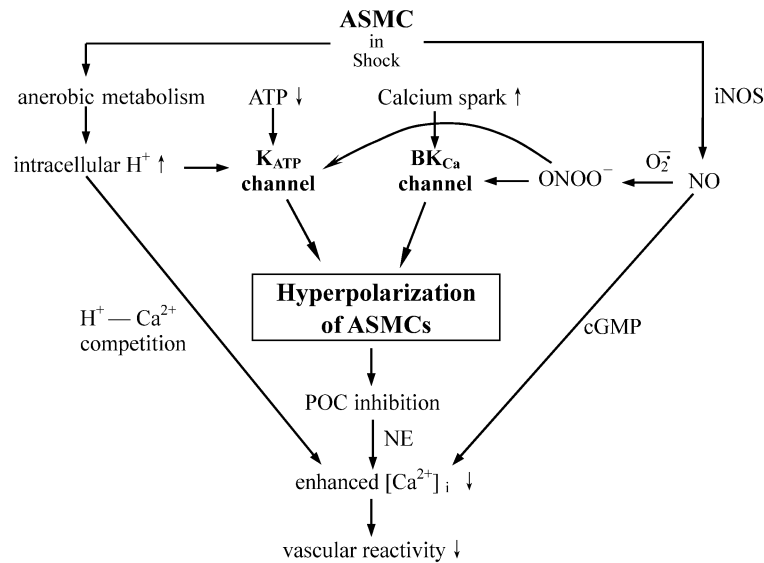


Fig. 13. The mechanisms of vascular hyporeactivity in severe shock [43]. K_{ATP} channel – ATP-sensitive potassium channel; BK_{Ca} channel – large conductance calcium activated potassium channel; POC – potential operated calcium channel.

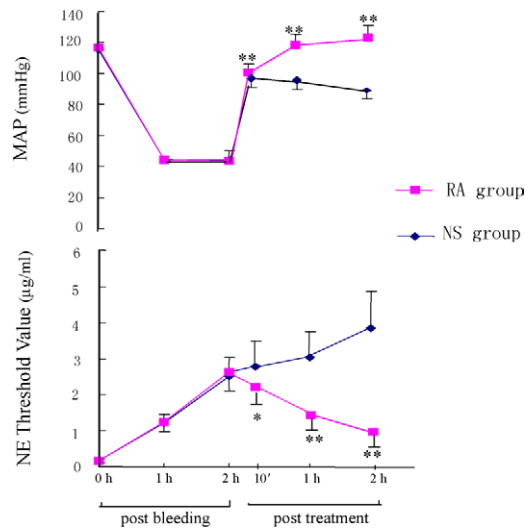


Fig. 14. The effect of restituting agents on BP and vasoreactivity of a rat in hemorrhagic shock [43]. RA group – the restituting agent group (glybenclamide and tiron). * $p < 0.05$, ** $p < 0.01$ compared to the NS groups.

(Table 7). In summary, the activation of the K⁺ channel with ASMC hyperpolarization is involved in the pathogenesis of vascular hyporeactivity in severe shock. Blockade of ASMC K⁺ channel activation followed by application of vasoconstrictors is a new approach to the treatment of refractory hypotension in irreversible shock [22,25,29,43].

Table 7
Survival rate of rats in hemorrhagic shock

	Body weight (g)	Blood loss (ml/100 g bw)	Survival time (h)	24 h survival rate
NS group	193.3 ± 16.0	2.7 ± 0.4	14.8 ± 5.9	1/8
RA group	197.0 ± 9.9	2.9 ± 0.6	26.7 ± 4.8**	7/8**
TI group	191.0 ± 7.5	2.7 ± 0.2	19.6 ± 4.9	2/8
GL group	201.0 ± 8.6	2.9 ± 0.2	26.9 ± 6.5**	4/8
DMSO group	196.0 ± 6.7	2.7 ± 0.3	16.6 ± 4.8	1/8

NS – normal saline; RA – restituting agent (glyb. + tiron); TI – tiron; GL – glybenclamide.

** $p < 0.01$ compared with NS or DMSO group.

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