

## NEW APPROACH TO TREATMENT OF SHOCK—RESTITUTION OF VASOREACTIVITY

Ke-seng Zhao, Xuliang Huang, Jie Liu, Qiaobing Huang, Chunhua Jin, Yong Jiang, Jianqiu Jin, and Guiling Zhao

Department of Pathophysiology, First Military Medical University, Guangzhou, 510515, China

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**ABSTRACT**—Our objective was to observe the therapeutic effect of restituting vasoreactivity agent in severe shock. A hemorrhagic shock (HS) model was reproduced in rat and the response of arterioles of spinotrapezius muscle to norepinephrine (NE) in HS was tested. The diameter, blood velocity, and volumetric flow in arteriole, and the mean arterial pressure (MAP) were measured. The therapeutic effect was observed after the treatment of restituting vasoreactivity agent (glybenclamide—an inhibitor of ATP sensitive potassium channel, and tiron—an oxygen free radical scavenger). The arteriolar vasoreactivity was significantly reduced with 15 fold increase of NE threshold 2 h post HS. After treated with restituting agent(RA), the vascular hyporeactivity of rat was apparently recovered, and the increased level of MAP following injection of dopamine was 1.8 times and 1.9 times more than that in NS-treated and DMSO-treated group respectively. After reinfusion of shed blood, the value of systemic blood pressure maintained more than 100mmHg and volumetric flow in arterioles in RA group were 2 times more than those in NS treated group within the 2h observation periods. The average survival time in RA treated group was also 1.8 times and 1.6 times longer than that in NS- treated and DMSO-treated group respectively. The restituting vasoreactivity agent is able to recover the lower vasoreactivity with excellent anti-shock effect in severe hemorrhagic shock.

**KEYWORDS**—Hemorrhagic shock, vascular smooth muscle reactivity, membrane potential, ATP-sensitive potassium channel, shock treatment

### INTRODUCTION

Vascular hyporeactivity leads to persistent hypotension and low perfusion which is one of the major causes of mortality in severe shock. However, there are few reports in the literature concerning the restitution of vasoreactivity in shock, as the mechanism of low vasoreactivity has not been elucidated completely (1–5). Recently, we have shown that membrane hyperpolarization of arteriolar smooth muscle cell (~ASMC) caused by activation of ATP-sensitive potassium channel ( $K_{ATP}$ ) is a major cause of low vasoreactivity in shock. Membrane hyperpolarization results in inhibition of potential operated calcium channel (POC) and reduction of  $Ca^{2+}$  influx, which in turn leads to reduction of the increased  $[Ca^{2+}]_i$  of ASMC stimulated by NE and the reduction of contractile response (6–9). Based on the above researches a new concept of restitution of vasoreactivity is proposed, which is a new approach to the treatment of severe shock by blocking  $K_{ATP}$  channel and prohibiting membrane hyperpolarization of ASMC. It was shown in isolated ASMC that glybenclamide, a selective blocker of the  $K_{ATP}$  channel, might decrease the level of ASMC hyperpolarization with the increase of NE-stimulated  $[Ca^{2+}]_i$  in the late stage of severe shock, and tiron, a scavenger of oxygen free radicals, might decrease the formation of peroxynitrite ( $OONO^-$ ) with the recovery of ASMC membrane potential. The purpose of the study is to see if glybenclamide and tiron can serve as restituting vasoreactivity agent *in vivo* in the treatment of rat with severe hemorrhagic shock.

### MATERIALS AND METHODS

#### *Preparation of shock model and determination of vascular reactivity*

Forty healthy Wistar rats, weighting 180–220g, were randomly divided into 5 groups:

1. saline-treated (NS) group
2. restituting agent (glybenclamide + tiron)-treated (RA) group.
3. tiron-treated (TI)group
4. Glybenclamide-treated(GL)group
5. DMSO-treated (DM)group (N = 8 for each group).

Rats were anesthetized with a mixture of 13.3% urethane and 1% chloralose  $\alpha$  (0.6 mL/100g body weight). Bilateral femoral arteries were cannulated with PE50 catheters for the measurement of mean arterial pressure (MAP) and blood withdrawal. The spinotrapezius muscle was isolated and prepared for microscopy using the technique described by Gray (10). The exposed muscle was suffused with balanced Krebs's solution (~NaCl 30.8g, KCl 1.4g,  $CaCl_2 \cdot 2H_2O$  1.16g,  $MgSO_4$  1.2g, dissolved in 400mL distilled water, the pH was adjusted to 7.4 with  $NaHCO_3$  at 37°) for controlling the temperature and pH constant and avoiding evaporative fluid loss of the exposed muscle. The image of the microcirculation was recorded using an Olympus microscope with a long working distance objective. The diameter of the arterioles was measured with a For A IV-550 video microscaler and a color TV set, and the velocity of blood cells ( $V_{rbc}$ ) was measured by a 102B RBC velocity tracking correlator (IPM Inc.). The volumetric flow of microvessels was calculated with the following formula:  $Q = (V_{rbc}/1.6) \cdot \pi \cdot D^2/4$  (11). The vasoreactivity to norepinephrine (NE) was determined by topical application of increasing concentrations of NE until a threshold concentration was found, which produced temporary contraction of the transverse arteriole at its branch point from the parent arcade that was reduced to half of its original diameter for 10–20 sec (12). The experiments were approved by the Animal Care Committee of the First Military Medical University and performed in adherence to National Institute of Health.

#### *The protocol of experimental therapy*

For each animal, blood was withdrawn from the femoral artery so as to lower the MAP until a stable level of 40–44 mmHg was reached. The blood was withdrawn into a 10 mL syringe in which the wall had been wetted with diluted heparin (1000 units/mL). Two hours after hemorrhage the rats were treated with different medicine: in RA group 0.2 mL of restituting agent (0.15 mg/kg glybenclamide in 0.1 mL DMSO and 300 mg/kg tiron in 0.1 mL NS) was given through the femoral vein,

Address reprint requests to Ke-seng Zhao, Department of Pathophysiology, The First Military Medical University, Guangzhou, 510515, China.

followed by 0.2 mL normal saline to rinse out the catheter; the same volume of normal saline, glybenclamide, tiron and DMSO was given in NS, GL, TI, and DM group respectively. After the measurement of vasoreactivity, dopamine (1 mg/kg) was slowly injected through the femoral vein, followed by 0.1 mL NS to rinse out the catheter. Then the shed blood was reinfused after the detection of MAP response to dopamine. The protocol consisted of a 20 min control period of observation, a 120 min period of hemorrhagic hypotension, and a 120 min period of recovery after giving restituting agents, dopamine, and reinfusion of shed blood.

Glybenclamide was dissolved first in DMSO (0.1 mmol/L), as a stored solution, and was diluted with Krebs's solution before use. The final concentration of DMSO was less than 0.2%. Tiron, 4,5-dihydroxy-1,3-benzene-disulfonic acid ( $C_6H_4O_8S_2Na_2$ )disodium salts, is an oxygen free radical scavenger and dissolved in normal saline.

At the end of 120 min microscopic observation, femoral arteries were ligated, catheters were removed, and the wound was sutured. The average survival time and 24 h survival rate were recorded.

The results are shown as mean value  $\pm$  standard deviation of the means. Differences between the means were evaluated for statistical significance by the Student's *t* test. Analysis of Variance (ANOVA) was used to determine significance of differences among groups. Survival statistics was carried out using Chi square analysis. A *P* value of  $< 0.05$  was considered as a significant difference.

## RESULTS

### General condition

There were no statistical difference of body weight and blood loss among the 5 groups ( $P > 0.05$ ). However, the average survival time of RA group and GL group was 1.8 times and 1.6 times longer than that of NS group and DM group respectively ( $P < 0.01$ ), and most of the rats (7/8) in RA group survived for 24 h, which was also much more than the rats (1/8) in NS group or DM group. The 24 h survival rate of RA group was higher than that of GL group ( $P < 0.05$ ), although the survival time was similar between the 2 groups. (Table 1).

### NE threshold value

The vasoreactivity to NE was apparently decreased in severe shock. The threshold concentration of NE increased to 15 times more than the prehemorrhage value 2 h post hemorrhage, increased to 17 fold soon after injection of 0.4 mL normal saline or DMSO, and to 25 fold 2 h post-treatment in NS group and DM group. Meanwhile in RA and GL group, the threshold concentration of NE also increased to 15 fold 2 h post-hemorrhage, but decreased to 13 fold 5 min after injection of drugs and decreased to 6.1 and 7.4 fold 2 h post treatment respectively (Table 2). The data indicated that the restituting agents can significantly restore the vasoreactivity in severe shock. In TI group, the NE threshold level was increased to 13.3 times more than prebleeding value 2 h post treatment, which was nearly half value of those in NS or DM group ( $P < 0.01$ ).

TABLE 1. Survival rate of rats with hemorrhagic shock

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

\*\* $P < 0.01$  compared with NS group or DM group.

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### The effect of dopamine on blood pressure

The rat was given various medicine 2h post-hemorrhage in different groups. After measuring the local vascular response of spinotrapezius muscle to NE stimulation, 0.1 mL of dopamine (1 mg/kg) was intravenously injected to observe if there was a good effect of dopamine on enhancing blood pressure with the improvement of vasoreactivity in severe shock. It was found that the injection of restituting agents caused mild increase of blood pressure in shock, leading MAP to rise from  $43.8 \pm 0.7$  mmHg to  $59.3 \pm 5.2$  mmHg in RA group. Meanwhile injection of 0.4 mL normal saline made MAP change only from  $44.0 \pm 0.8$  to  $49.3 \pm 2.3$  mmHg, which was less than the change in RA group ( $P < 0.05$ ).

After injection of dopamine, the value of MAP was increased by  $27.0 \pm 2.3$  and  $25.2 \pm 4.9$  mmHg in RA group and GL group respectively (Table 3), which were much higher than  $15.0 \pm 4.1$  and  $14.2 \pm 3.9$  mmHg in NS group and DM group respectively ( $P < 0.01$ ). However, the increased value of MAP in TI group was  $16.1 \pm 4.1$  mmHg, which was no significant difference from NS and DM group.

### The relationship between blood pressure and vasoreactivity

It was shown in Figure 1 that the MAP value at each time point after treatment in RA group was much higher than that in NS group. Although MAP increased to  $97.5 \pm 4.5$  mmHg 5 min after reinfusion of shed blood, it gradually decreased to  $88.3 \pm 6.1$  mmHg 2 h post treatment in NS group. Meanwhile, MAP increased to  $107.5 \pm 5.4$  mmHg 5 min after reinfusion of shed blood and maintained at  $116.6 \pm 7.7$  mmHg 2 h post treatment in RA group, which was much higher than that in NS group ( $P < 0.01$ ).

It was shown in Figure 1 that the alteration of vasoreactivity was in concordance with the change of MAP and the correlation coefficient was 0.89 ( $P < 0.01$ ). The result indicated that the restitution of vasoreactivity led to blood pressure increased steadily after treatment of severe hemorrhagic shock (Fig. 1).

### Change of microcirculation

There was no apparent change of transverse arteriole (A3) diameter after given restituting agents. Volumetric flow was drastically reduced in arterioles by over 90% in both NS group and RA group, coincident with the induced hypotension following hemorrhage. However, with the increase of blood pressure at each time point after treatment the A3 arteriole blood velocity and blood volume in RA group were much higher than those in NS group ( $P < 0.01$ ). Two hours post-treatment the blood velocity of A3 arteriole was  $3.4 \pm 0.8$  mm/s in RA group, which was about 2 times of the velocity ( $1.7 \pm 0.3$  mm/s) in NS group ( $P < 0.01$ ). Meanwhile the volumetric flow of A3 arteriole in RA group was  $1093.8 \pm 276.5$  pl/s, which was also 2 times of the volume ( $512.7 \pm 109.9$  pl/s) in NS group (Fig. 2).

## DISCUSSION

Vascular hyporeactivity is one of the major causes responsible for low blood pressure which is almost irreversible and was refractory to vasopressor drugs or fluid resuscitation in

TABLE 2. Changes of vasoreactivity in hemorrhagic shock

Group	Threshold concentration of NE to contract A3 arteriole ( $\mu\text{g/ml}$ )			
	Prebleeding	2 h post bleeding	5 min post giving drug	2 h post treatment
NS	0.16 $\pm$ 0.06	2.50 $\pm$ 0.13	2.70 $\pm$ 0.53	4.14 $\pm$ 0.90
RA	0.17 $\pm$ 0.04	2.60 $\pm$ 0.51	2.10 $\pm$ 0.53*	1.05 $\pm$ 0.27*
G1	0.16 $\pm$ 0.05	2.62 $\pm$ 0.53	2.16 $\pm$ 0.42*	1.19 $\pm$ 0.26*
TI	0.16 $\pm$ 0.05	2.69 $\pm$ 0.25	2.76 $\pm$ 0.55	2.13 $\pm$ 0.34*
DM	0.16 $\pm$ 0.05	2.57 $\pm$ 0.56	2.72 $\pm$ 0.56	4.14 $\pm$ 0.89

\* $P < 0.05$  vs. NS group or DM group.

TABLE 3. The effect of restituting vasoreactivity agent on MAP in shock

	MAP (mmHg)			
	Prebleeding	2 h post-bleeding	Soon after giving medicine	5 min after giving dopamine
NS group	115.7 $\pm$ 4.7	44.0 $\pm$ 0.8	49.3 $\pm$ 2.3	64.3 $\pm$ 5.7
RA group	116.5 $\pm$ 4.8	43.8 $\pm$ 0.7	59.3 $\pm$ 5.2*	86.3 $\pm$ 6.6**
TI group	112.1 $\pm$ 3.9	42.0 $\pm$ 1.0	54.6 $\pm$ 9.6	70.6 $\pm$ 5.7
GL group	112.6 $\pm$ 6.3	42.8 $\pm$ 1.8	51.4 $\pm$ 3.9*	76.6 $\pm$ 7.0**
DM group	112.5 $\pm$ 5.4	41.8 $\pm$ 1.2	43.3 $\pm$ 7.9	57.5 $\pm$ 9.3

\* $P < 0.05$ , \*\* $P < 0.01$  vs the value before giving medicine and the corresponding value in NS group or DM group.

severe shock. Accumulation of metabolites, desensitization of adrenergic receptor, and harmful effect of nitric oxide were thought to contribute to vascular hyporeactivity, but the molecular mechanism responsible for these changes are still unclear (13–18).

The potassium channels of vessel smooth muscle were reported to be closely related with the vascular response to vasoactive agent (6, 7, 19, 20–22). Based on our previous works, we put forward a scheme of ASMC hyperpolarization in the pathogenesis of low vasoreactivity in shock (Fig. 3). Prevention of ASMC hyperpolarization is possible and may result in recovery of low vasoreactivity in shock. It was shown in isolated ASMC that to block  $K_{ATP}$  channel by glybenclamide, to abolish formation of peroxynitrite ( $\text{OONO}^-$ ) by tiron, and to neutralize intracellular acidosis by  $\text{NaHCO}_3$  could reverse or attenuate the hyperpolarization of ASMC in severe hemorrhagic shock. However, it is not possible to observe vessel contractile response in isolated ASMC. So we should do animal experimentation *in vivo* to answer 3 questions, i.e., if it could restore vasoreactivity to prevent ASMC hyperpolarization, if it could enhance blood pressure to give restituting agent followed by vasoconstrictor agent, and if it could finally increase the survival rate in severe shock. For simplification, 2 agents (glybenclamide and tiron) were used in this study, the action point of which could be seen in Figure 3.

It is difficult to quantify vasoreactivity *in vivo* during shock, as desensitization of adrenergic receptor, NO, and other events may appear and influence the measurement. However, the interesting thing in the study is the final extent of vasoreactivity alteration, despite various reasons. The local NE stimulation method was used in the study, as the contractile response of arteriole in exposed muscle could be seen under microscopy. It was shown that treatment of restituting agents really could reconstitute vasoreactivity in severe shock. The value of NE

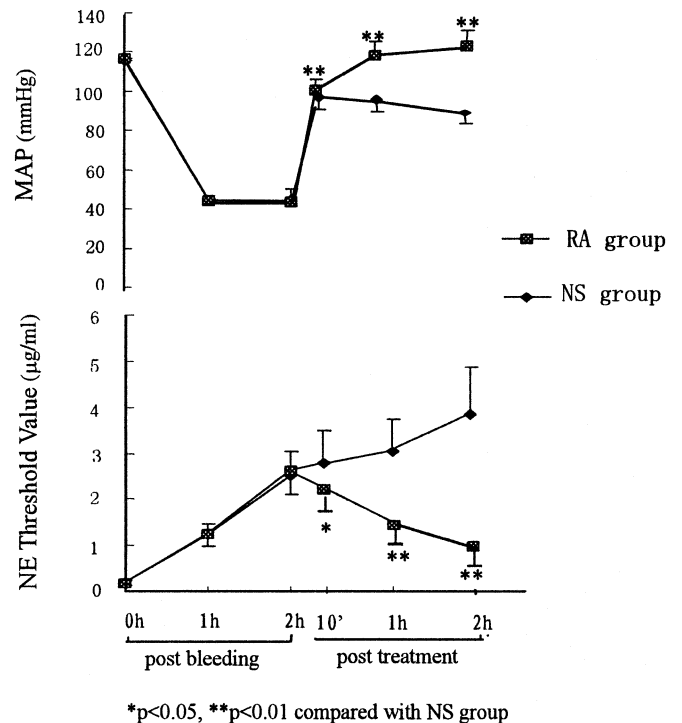


FIG. 1. The effect of restituting vasoreactivity agent (glybenclamide and tiron) on the blood pressure and vasoreactivity of rat in hemorrhagic shock. The treatment includes injection of glybenclamide, tiron, dopamine, and reinfusion of shed blood in test group (RA group); and normal saline, dopamine, and shed blood in control group (NS group). Plot shows that with the recovery of vasoreactivity, MAP value at each time point after treatment is much higher than that in NS group.

threshold concentration increased to 15 times more than the prehemorrhage value 2 h after hemorrhage in different groups. However, the trend of vasoreactivity after treatment was different between the control groups and treated groups. The NE threshold value was still increasing after given normal saline or DMSO, dopamine, and shed blood, and reaching to 25 times at 2 h post treatment in NS and DM group. However the NE threshold value fell down soon after giving restituting agents and decreased from 15 times to 6.1 and 7.4 times 2 h after treatment in RA and GL group respectively. With the recovery of vasoreactivity, the effect of dopamine on the blood pressure in RA group was 1.8 and 1.9 times as much as that in NS or DM 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. It was shown in microcirculation observation that the volumetric flow in the arteriole at each time point after treatment was significantly increased in RA group, which reached to 2 times more than

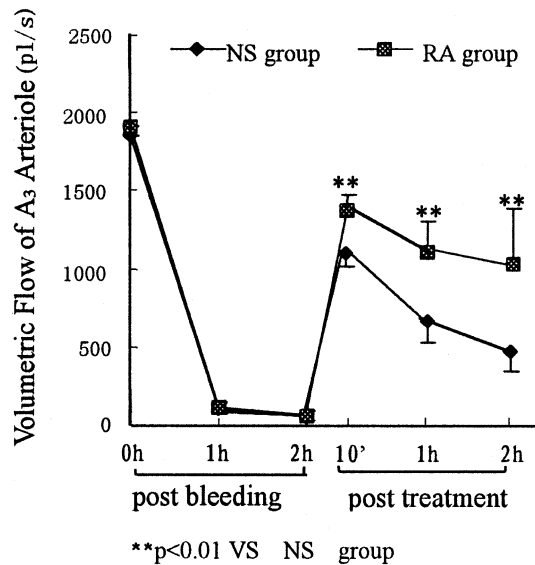


FIG. 2. The effect of restituting vasoreactivity agent on the volumetric flow of rat A<sub>3</sub> arteriole in shock. Plot shows that the volumetric flow of A<sub>3</sub> arteriole in test group (RA group) is 2 times more than that in control group (NS group) 2 h post treatment.

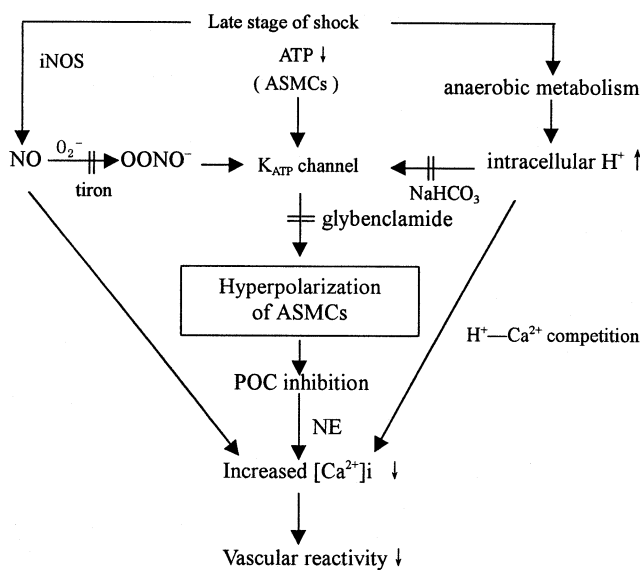


FIG. 3. The scheme of vascular hyporeactivity mechanism in severe shock. Plot shows the action point of the restituting agents (glybenclamide, tiron, and NaHCO<sub>3</sub>). POC—potential operated calcium channel.

that in NS group 2 h post treatment. With the increase of vital organ perfusion, the survival rate of RA group was also increased including 7 of 8 rats survived for 24 h, which was much higher than 1 of 8 rats in NS or DM group. The increased volumetric flow of microcirculation post treatment was caused by increased blood velocity, which was related to the elevation of blood pressure, as there was no significant change of microvessel diameter after injection of the restituting agent. These indicated that restitution of vasoreactivity, which led to enhance of blood pressure, had important significance in the treatment of severe shock. The study also indicated that simple tiron treatment could partially recover the low vasoreactivity with no significant increase of survival time in shock, and simple glybenclamide treatment could lead to enhance of

survival time with lower 24 h survival rate. Treatment of glybenclamide combined with tiron could result in the best therapeutic effect on severe hemorrhagic shock.

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