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Original Article

Polyethylene-oxide improves microcirculatory blood flow in a murine hemorrhagic shock model

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Abstract: Background: Polyethylene oxide (PEO) is a synthetic polymer commonly used in medicine production to reduce toxicity. In the present study, we assessed whether PEO can have a functional effect on improving microcirculatory blood flow after hemorrhagic shock in an animal model. Methods: Hemorrhagic shock (HS) was introduced in 78 C57BL/6 mice, which were then equally divided into two groups. One group of mice was intravenously injected with PEO (diluted in Ringer's solution (RS), PH = 7.4), and the other with RS only. The parameters of microcirculatory hemodynamics, arterial blood gas analysis and multi-organ functions were compared between two groups, 0, 3, 12 and 24 hours after resuscitation. Results: After HS, the hemodynamics, including microvascular diameter, red blood cell velocity, and blood flow rates were significantly improved in time-dependent manners in PEO treated mice. Most parameters of arterial blood gas analysis, except PCO_2, were also significantly improved by PEO. Multi-organ immunohistochemistry demonstrated that congestions and inflammatory responses in liver and lung were markedly ameliorated by PEO. Conclusions: Our results demonstrated that PEO infusion could effectively improve microcirculation after hemorrhagic shock and increase the chance of survival in animal models.

Keywords: PEO, multiple organ dysfunctions, hemorrhagic shock

Introduction

Worldwide, traumatic injury is one of the leading cause of deaths in individuals from ages 5 to 44 [1]. In some of the worst scenarios, trauma and then induced hemorrhagic shock (HS) can result in hemorrhage-related deaths in as quickly as 6 hours after the onset of trauma [2, 3]. Even in patients survived from initial trauma, the abnormality in microcirculation may significantly increase the chance for them to develop post-shock multiple organ dysfunction syndrome (MODS) [4]. The common treatments for HS are suppressing bleeding and expanding blood volume by fluid resuscitation [5, 6]. However, clinical interventions to efficiently reduce the risk of multiple-organ dysfunctions are largely lacking.

Polyethylene oxide (PEO) is one type of biocompatible water-soluble polymeric prodrugs that have been extensively used in medical applications in recent decades [7, 8]. Notably, several advantages could be taken while applying polymeric prodrugs, including increasing solubility of lipophilic drugs, preventing drug from early degradation and prolonging drug circulation period in human body [9, 10]. Interestingly, it has been shown in the cardiac microcirculation system that polymeric prodrugs (including PEO) may have profound therapeutic effects. For example, drag-reducing polymers was shown to reduce the blood pressure loss between the aorta and the arterioles [11], improve microcirculation after acute peripheral ischemia [12] and restore the hemodynamic functions after myocardial infarction [13]. Unfortunately, the underlying molecular mechanisms for PEO to improve cardiovascular functions are largely unknown.

In the current study, we established a murine model of hemorrhagic shock, and then intravenously administrated PEO or control saline into after-shock mice. The possible biological effects of PEO, as compared with control, on improving after-shock microcirculation and reducing multiple organ dysfunctions were assessed.

Materials and methods

Animals

Male C57BL/6 mice, age 4-8 weeks, were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. The animals were then maintained at the Animal Care Facility at the First Affiliated Hospital of Zhengzhou University with a 12/12 hours light/dark cycle and full access to standard laboratory feed and water. The protocols of current study followed the guidelines for the use of experimental animals of the US National Institutes of Health, and were approved by the Animal Research and Ethic Committee at the First Affiliated Hospital of Zhengzhou University in Zhengzhou, China.

Preparation of PEO

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. PEO, with molecular weight of 5,000 KDa, was diluted in 0.9% physiological saline to achieve stock concentration of 5 mg/mL followed by 6-day dialyzation by passing through a filter with a molecular weight cut-off of 50 kDa (Amicon, USA). Eight hour prior experiment, PEO stock solution was further diluted with normal saline to a final concentration of 50 mg/mL.

Hemorrhagic shock model

Mice were anesthetized with xylazine (20 mg/kg) and ketamine (100 mg/kg), and maintained in a supine position. The right carotid artery was cannulated with PE-10 tubing and attached to a pressure transducer (WPI, USA) for blood pressure monitoring. The right femoral artery was cannulated with another PE-10 tubing for blood withdrawal and administration of the resuscitation fluid. To establish the Hemorrhagic shock (HS) model, mice were initially given a 45-min period to stabilize hemodynamic parameters, followed by withdrawing blood from the femoral artery into a heparinized syringe for 30 min (1 μl/g/min) to reach a blood pressure of 45 mmHg. Mice were then closely monitored for another 60 mins. If necessary, additional blood withdrawal was conducted to maintain 45 mmHg blood pressure.

Resuscitation and PEO administration

After the establishment of HS, mice were randomly divided into two groups, and were resuscitated with sterile isotonic solutions, either mixed with PEO or Ringer solution (RS), through the femoral artery catheter, equaling to two times of the total volume of shed blood, in 2 hours. The amount of PEO was determined by following equation, [body weight (grams) × 0.05]/10 ml.

Microhemodynamic parameters assessment

The protocol to assess microhemodynamic parameters was conducted according to the method described in a previous study [14]. Briefly, dorsal facet of the left side spinotrapezius muscle was exposed. Microvessels and adjacent connective tissues were observed on a Zeiss inverted microscope with a ×68 water-immersion objective (Zeiss, Germany), and were recorded by a video camera (Zeiss, Germany). Microvessels were classified according to their diameters (≤ 30 mm) and their positions within the microvascular network. Microvascular diameter (D) and red blood cell (RBC) velocity (V_{RBC}) were measured on-line, and blood flow rates (Q) were calculated according to the formula, $Q = V_{RBC}/1.6 \times D^2/4$, 0, 3, 12 and 24 hours after resuscitation.

Arterial blood gas assessment

Mice were also sacrificed 0, 3, 12 and 24 hours after resuscitation. A total volume of 1 mL blood samples from each mouse was used to assess the parameters of arterial blood gas, including blood pH, hemoglobin concentration (Hgb), base excess, HCO_3^-, PO_2, PCO_2 and lactate, by a Hitachi 8500 automatic analyzer (Hitachi, Japan).

Immunohistochemistry

Upon sacrifice, liver and lung samples were quickly fixed in 4% formaldehyde solution and then embedded in paraffin. Cross sections (~12 μm thick) were prepared and immunostained with hematoxylin-eosin (H & E), followed by microscopic examination.

Statistical analysis

All data were presented as Means ± S.E.M. Comparisons between groups were performed by one-way ANOVA complimented with LSD test, or Student's t-test. Differences were considered significant if $P < 0.05$. All experiments were repeated at least three times.

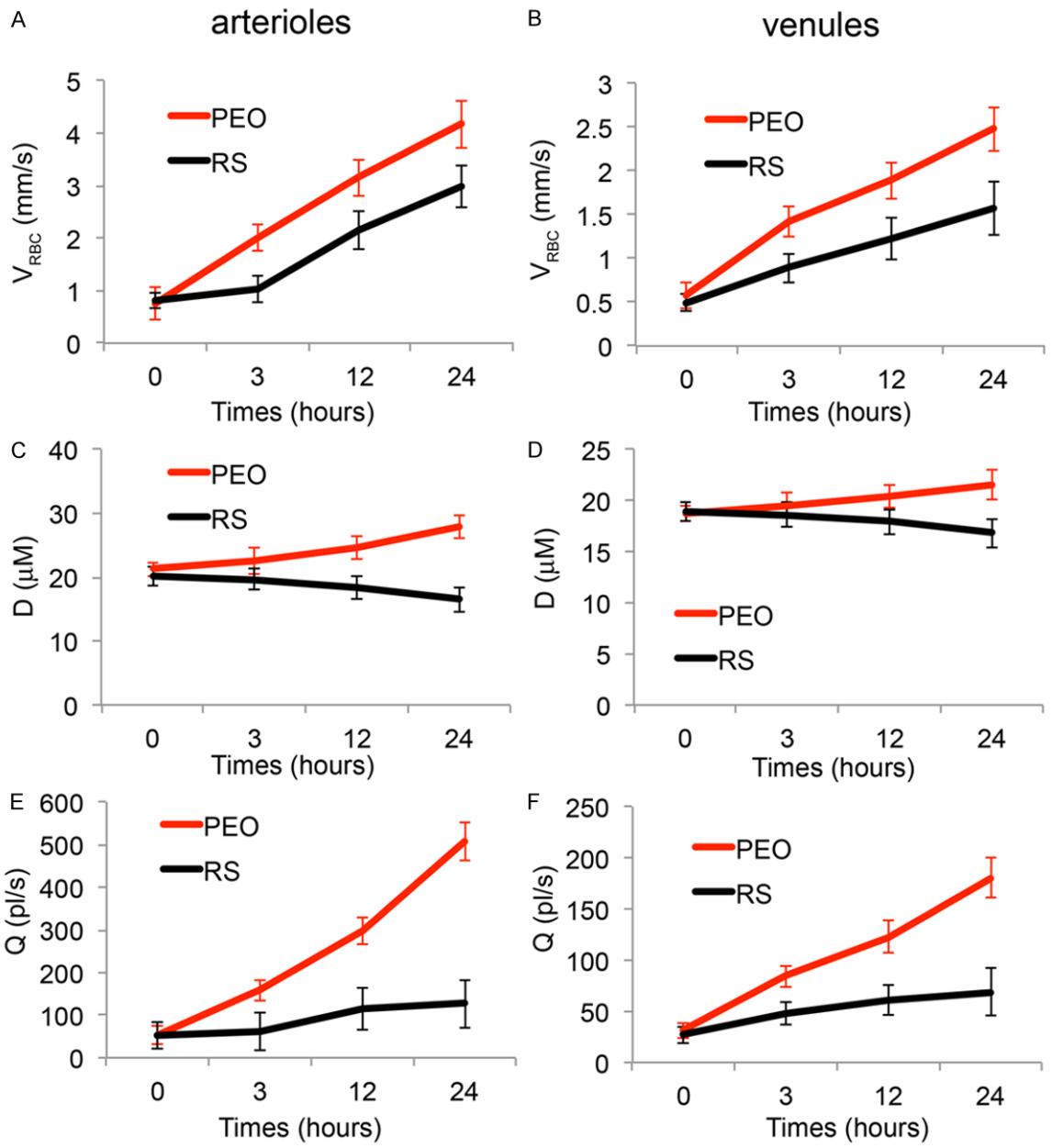

Figure 1. PEO improved microcirculation after hemorrhagic shock. Hemorrhagic shock was introduced in a mouse model, followed by induction of PEO, and vehicle control of Ringer Solution (RS). At different time points after drug induction, including 0, 3, 12 and 24 hours, red blood cell (RBC) velocity (V_{RBC}) (A, B), microvascular diameter (D) (C, D) were measured in both arterioles and venules. Subsequent blood flow rates (Q) were calculated according to the formula, $Q = V_{RBC}/1.6 \times D^2/4$ (E, F) ($P < 0.05$, one-away ANOVA).

Results

PEO improved microhemodynamics after hemorrhagic shock in mice

Firstly in the present study, we introduced hemorrhagic shock in a mouse model. During resuscitation after shock, PEO was introduced in after-shock mice. The control mice were induced with Ringer solution (RS) only. The microhemodymaics in the mice, V_{RBC} and D were recorded from both micro- arterioles and venules, at 0, 3, 12 and 24 hours after resuscitation. We discovered that, in arterioles and venules, V_{RBC} (**Figure 1A**, **1B**) and D (**Figure 1C**, **1D**) were significantly increased in time-depen-

Table 1. Blood gas analysis in mice induced with PEO or RS, 24 hours after resuscitation

Properties	RS (n = 12)	PEO (n = 14)	*P* value
PH	7.11 ± 0.04	7.30 ± 0.05	0.021
PO_2 (mmHg)	71.5 ± 2.4	88.7 ± 2.1	0.003
PCO_2 (mmHg)	38.4 ± 1.4	38.1 ± 2.0	0.071
HCO_3 (mmol)	17.5 ± 0.5	22.4 ± 1.0	0.004
LAC (mM)	3.6 ± 0.2	2.5 ± 0.4	0.010
BE (mEq/L)	-7.5 ± 1.0	-3.6 ± 0.9	0.004
Hgb (g/dL)	122.18 ± 3.5	145.8 ± 2.1	0.001

Abbreviations: RS, ringer solution; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; HCO_3^-, bicarbonate level; LAC, lactate concentration; BE, base excess; Hgb, hemoglobin concentration.

dent manners in PEO group, as compared to the values in RS group ($P < 0.05$, one-way ANOVA). As a result, the blood flow rates (Q) in both arterioles and venules were subsequently increased dramatically in mice induced with PEO, than in mice induced with RS only (**Figure 1D**, **1E**, $P < 0.05$, one-way ANOVA). Thus, our results demonstrated that microcirculation during resuscitation was significantly improved by PEO.

PEO improved arterial blood gas after hemorrhagic shock in mice

Since our results showed that PEO induction significantly improved microcirculation in mice after hemorrhagic shock, we then investigated whether PEO had any effect on arterial blood gas (ABG). For that aim, we evaluated many properties of ABG 24 hours after resuscitation and drug induction. Our results showed that, in mice induced with PEO, many of the blood gas properties were much improved (**Table 1**). For example, PH value was improved from 7.11 ± 0.04 in RS group to 7.30 ± 0.05 in PEO group ($P = 0.021$). Partial pressure of oxygen (PO_2) was increased from 71.5 ± 2.4 mmHg in RS group to 88.7 ± 2.1 mmHg in PEO group ($P = 0.003$), and bicarbonate level (HCO_3) was increased from 17.5 ± 0.5 mmol in RS group to 22.4 ± 1.0 mmol in PEO group ($P = 0.004$). Noticeably, partial pressure of carbon dioxide (PCO_2) was not different, 38.4 ± 1.4 mmHg in RS group and 38.1 ± 2.0 in PEO group ($P = 0.071$).

PEO improved multi-organ functions after hemorrhagic shock in mice

Finally, we used histochemical method to examine the effect of PEO on multi-organ functions in mice after hemorrhagic shock (**Figure 2**). As expected, in RS control group, substantial liver congestion and lung inflammation were seen by H & E staining (arrows). However, in mice treated with PEO, the liver and lung tissues were much smooth and the congestive and inflammatory responses were significantly ameliorated (**Figure 2**, bottom panel).

Discussions

In the present study, we examined whether the application of PEO could improve resuscitation after animals suffer a severe hemorrhagic shock in a mouse model. We demonstrated that the hemodynamic parameters, including microvascular diameter, red blood cell velocity, and blood flow rates were significantly improved in time-dependent manners in mice induced with PEO, as compared to the mice induced with control saline of PS. Also, arterial blood gas analysis showed that in mice induced PEO, the majority of the hematologic parameters (barring PCO_2) were significantly improved. Finally, immunohistochemistry of liver and lung tissues on mice 24 hours after resuscitation demonstrated that, hemorrhagic shock induced organ congestions and inflammation were markedly ameliorated by PEO.

It was shown in a previous study that drug-reducing polymers improved micro-vascular perfusion during coronary stenosis, possibly by a mechanism of reducing the pressure drop between aorta and arterioles [11]. This hypothesis was then confirmed by the results in our study, as we showed that V_{RBC} and D in arterioles were both increased by PEO (**Figure 1**). Thus, it is very likely that the mechanism of PEO to increase capillary blood flow is not only induced by sheer vasodilation, but also by improvements on hydrodynamics directly affected by PEO. However, as shown in other literatures, the relieving effect of PEO might be temporary as systemic failures, such as mitochondrial dysfunction may reduced the chance of full resuscitation after hemorrhagic shock [15, 16].

An encouraging observation of our study is that, the beneficial effects of PEO can last as long as 24 hours after hemorrhagic shock. It was shown in the literature that drug-reducing polymers degraded quickly, especially in blood contents, within an hour after initial induction

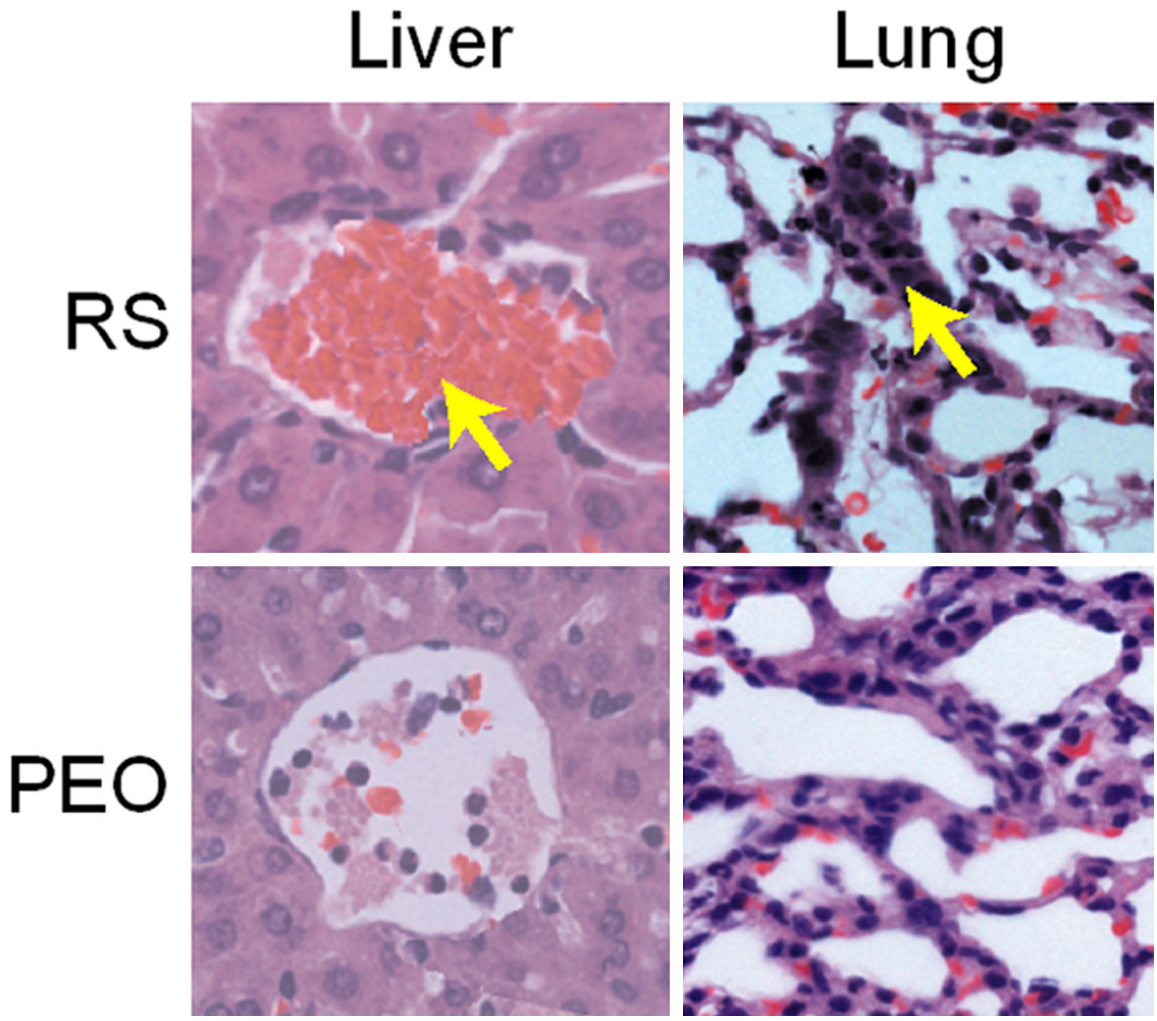

Figure 2. PEO improved organ functions after hemorrhagic shock. Twenty four hours after drug induction, mice were sacrificed and H & E staining was performed on liver and lung tissues. In RS group, hemorrhagic shock induced liver congestions and lung inflammation (arrows). However, in PEO group, the congestive and inflammatory responses were significantly ameliorated.

[17]. The reason for fast degradation may be partially, if not completely due to dissociation of molecular structures aggregates in the solution [17, 18]. However, it was very difficult to explore the time course of PEO degradation in our study, as most of the experiments were conducted *in vivo*. For that reason, we did not have exclusive evidence to explain whether the improvements on microcirculation or the de-inflammatory and de-congestive effects on organ function were directly induced by PEO, or by delayed systemic reactions after PEO dissociation. Thus, future experiments with the application of fluorescence-tagged drug-reduced polymer may help to solve this puzzle.

In conclusion, our study demonstrated that PEO could improve microcirculation in a mice model of severe hemorrhagic shock. Therefore, PEO may be a potential therapeutic approach for the treatment of patients suffered from acute hemorrhagic anemia after trauma.

Disclosure of conflict of interest

None.

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