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Resuscitation Fluid with Drag Reducing Polymer Enhances Cerebral Microcirculation and Tissue Oxygenation after Traumatic Brain Injury Complicated by Hemorrhagic Shock

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Abstract

Traumatic brain injury (TBI) is frequently accompanied by hemorrhagic shock (HS) which significantly worsens morbidity and mortality. Existing resuscitation fluids (RF) for volume expansion inadequately mitigate impaired microvascular cerebral blood flow (mvCBF) and hypoxia after TBI/HS. We hypothesized that nanomolar quantities of drag reducing polymers in resuscitation fluid (DRP-RF), would improve mvCBF by rheological modulation of hemodynamics.

Methods: TBI was induced in rats by fluid percussion (1.5 atm, 50 ms) followed by controlled hemorrhage to a mean arterial pressure (MAP) = 40 mmHg. DRP-RF or lactated Ringer (LR-RF) was infused to MAP of 60 mmHg for 1 h (pre-hospital), followed by blood re-infusion to a MAP = 70 mmHg (hospital). Temperature, MAP, blood gases and electrolytes were monitored. *In vivo* 2-photon laser scanning microscopy was used to monitor microvascular blood flow, hypoxia (NADH) and necrosis (i.v. propidium iodide) for 5 h after TBI/HS followed by MRI for CBF and lesion volume.

Results: TBI/HS compromised brain microvascular flow leading to capillary microthrombosis, tissue hypoxia and neuronal necrosis. DRP-RF compared to LR-RF reduced microthrombosis, restored collapsed capillary flow and improved mvCBF ($82 \pm 9.7\%$ vs. $62 \pm 9.7\%$, respectively, $p < 0.05$, $n = 10$). DRP-RF vs LR-RF decreased tissue hypoxia ($77 \pm 8.2\%$ vs. $60 \pm 10.5\%$, $p < 0.05$), and neuronal necrosis ($21 \pm 7.2\%$ vs. $36 \pm 7.3\%$, respectively, $p < 0.05$). MRI showed reduced lesion volumes with DRP-RF.

Conclusions: DRP-RF effectively restores mvCBF, reduces hypoxia and protects neurons compared to conventional volume expansion with LR-RF after TBI/HS.

1 Introduction

Traumatic brain injury (TBI) is frequently accompanied by arterial hypotension resulting from hemorrhagic shock (HS) which significantly worsens neurologic outcome, and increases mortality [1]. The main reason for this is increased severity of reduced cerebral blood flow (CBF) leading to capillary microthrombosis, hypoxia, neuronal death and two-fold increase in contusion volume [2]. In TBI/HS, arterial pressure reductions, normally well-tolerated, causes severe reductions in CBF and increased ischemia even after mild TBI [2]. We previously showed that even in a healthy rat brain, decreasing arterial pressure to 40

mmHg for 1 h compromised cerebral microcirculation and CBF autoregulation leading to tissue hypoxia [3,4].

Current resuscitation fluids (RF) for volume expansion after TBI/HS do not adequately ameliorate impaired microvascular cerebral blood flow (mvCBF). In our previous studies in a rat TBI model, we have shown that nanomolar concentrations of intravascular blood soluble drag reducing polymers (DRP) significantly enhanced microvascular perfusion and tissue oxygenation in peri-contusional areas and protected neurons [5]. It has been also demonstrated that DRP improved hemodynamics, blood chemistry, and survival in various animal models of HS [6]. Hence, we *hypothesized* that the addition of nanomolar quantities of DRP to resuscitation fluids would improve mvCBF and tissue oxygenation by rheological modulation of hemodynamics. We compared the effects of lactated Ringer resuscitation fluid with DRP (DRP-RF) and without (LR-RF) on mvCBF, tissue oxygenation and neuronal survival in the rat brain after TBI and HS.

2 Methods

Most of the procedures used in these studies we described previously [5]. Protocol #200640 was approved by the Institutional Animal Care and Use Committee of the University of New Mexico and the studies were conducted according the NIH Guide for the Care and Use of Laboratory Animals.

Surgical preparation.

Laboratory-acclimated male Sprague-Dawley rats (250–300 g) were mechanically ventilated on isoflurane (2%), nitrous oxide (69%) oxygen anesthesia (29%). Femoral vein and artery catheters were inserted. For imaging and TBI, a 5-mm craniotomy over the left parietal cortex was filled with 2% agarose in saline and sealed by cover glass. The fluid percussion was used as a model of TBI and was induced by 1.5 atm 50 ms pulse from a custom-built Pneumatic Impactor connected to the brain through a pressure transducer filled with artificial cerebrospinal fluid. HS was performed in a way similar to that described by Robertson et al. [2].

Overall design of the study.

TBI was induced after baseline in-vivo 2-photon laser scanning microscopy (2PLSM) and followed by a 1-h hemorrhagic phase, where blood was slowly withdrawn through the femoral vein to reduce mean arterial pressure (MAP) to 40 mmHg. In the following 1-h pre-hospital care phase, resuscitation fluids (LR-RF or DRP-RF) were slowly infused i.v. to raise MAP to ~55 mmHg and CBF to ~65% of baseline. In a subsequent 3-h definitive hospital care phase, shed blood was re-infused to a MAP of 70 mmHg and CBF of ~75% of baseline. *In vivo* 2PLSM was done throughout the study over the peri-contusion area of the parietal cortex of the rat brain. Monitored variables included: cerebral microvascular blood flow velocity, number of perfused capillaries, tissue oxygenation (NADH) and neuronal necrosis (i.v. Propidium Iodide). The laser Doppler flux was measured via a lateral temporal window using a 0.9 mm diameter probe (DRT4, Moor Inst., Axminster, UK) in the same region of the brain studied by 2PLSM. Brain and rectal temperatures were monitored and maintained

at $38 \pm 0.5^{\circ}\text{C}$. Arterial blood gases, electrolytes, hematocrit and pH were measured hourly (epoc Blood Analysis System, Alere Inc., Waltham, MA, USA).

DRP preparation.

Polyethylene oxide (PEO, MW ~4000 kDa) was dissolved in saline to 0.1% (1000 ppm), dialyzed against saline using a 50 kD cutoff membrane, diluted in saline to 50 ppm, slow rocked for ~2 hours and then sterilized using a $0.22\text{ }\mu\text{m}$ filter [6]. DRP-RF was prepared before infusion by adding DRP to Lactated Ringer to reach final DRP concentration of 0.0005% (5 ppm).

Two-Photon Laser Scanning Microscopy.

Fluorescent serum (i.v. fluorescein isothiocyanate (FITS) dextran, 150 kDa in physiological saline, 5% wt/vol) was visualized using an Olympus BX 51WI upright microscope and water-immersion LUMPlan FL/IR 20X/0.50 W objective. Excitation was provided by a PrairieView Ultima multiphoton microscopy laser scan unit powered by a Millennia Prime 10 W diode laser source pumping a Tsunami Ti: Sapphire laser (Spectra-Physics, Mountain View, CA, USA) tuned to 750 nm centre wavelength. Band-pass-filtered epifluorescence (510–550 nm for FITS and 425–475 nm for NADH) was collected by photomultiplier tubes of the Prairie View Ultima system. Images (512×512 pixels, $0.15\text{ }\mu\text{m}/\text{pixel}$ in the x- and y-axes) or line scans were acquired using Prairie View software. Red blood cell flow velocity was measured in microvessels ranging from $3\text{--}50\text{ }\mu\text{m}$ diameter up to $500\text{ }\mu\text{m}$ below the surface of the parietal cortex, as described previously [5]. Tissue hypoxia was assessed by measurement of NADH autofluorescence. In offline analyses using NIH ImageJ software, three-dimensional anatomy of the vasculature in areas of interest were reconstructed from two-dimensional (planar) scans of the fluorescence intensity obtained at successive focal depths in the cortex (XYZ stack). To evaluate *neuronal survival*, $200\text{ }\mu\text{L}$ of a propidium iodide (PI)/saline, which labels only necrotized cells with damaged membrane, was injected intravenously during surgical preparation. Propidium iodide fluorescence was band-pass filtered at 600–620 nm (as described previously) to visualize damaged neurons [7].

Statistical analyses were done using GraphPad Prism software 6.0 (La Jolla, CA, USA) by Student's t-test or Kolmogorov-Smirnov test where appropriate. Differences between groups were determined using two-way analysis of variance (ANOVA) for multiple comparisons and post-hoc testing using the Mann-Whitney U-test.

3 Results

TBI did not cause notable changes in MAP, while cortical CBF, measured by surface laser Doppler probe in the peri-contusional area, fell to $87.5 \pm 6.2\%$ from baseline ($P < 0.05$). In the peri-contusion area after TBI, microvascular CBF, measured by *in-vivo* 2PLSM showed a reduction in capillary flow velocity to $0.59 \pm 0.03\text{ mm/s}$ from $0.77 \pm 0.05\text{ mm/s}$ and about ~25% reduction in the number of functioning capillaries due to microthrombosis leading to tissue hypoxia, reflected by a ~25% increase in NADH autofluorescence (Fig. 1; $P < 0.05$ from baseline) which agrees with our previous observations [5].

Subsequent hemorrhagic shock reduced MAP to 40.2 ± 3.7 mmHg and cortical CBF to $45 \pm 7.1\%$, which caused a further two-fold reduction of mvCBF and tissue oxygenation leading to neuronal necrosis with 59.6 ± 6.1 dead neurons per 0.075 mm^3 at the end of the HS phase (Figs. 1, 2; $P < 0.05$ from TBI).

In the pre-hospital phase, conventional LR-RF, slowly infused in the amount of 5.4 ± 2.1 ml, restored MAP to 55 ± 5.4 mmHg and cortical CBF to $62.8 \pm 7.2\%$ ($P < 0.05$ from the HS phase). However, no notable changes were observed in mvCBF and tissue oxygenation which remained at the same level as in the HS phase (Fig. 1), while the number of dead neurons increased to 138.3 ± 7.6 per 0.075 mm^3 of brain parenchyma (Fig. 2, $P < 0.05$ from the HS phase).

DRP-RF, infused during *the pre-hospital phase* in the smaller amount of 2.1 ± 0.3 ml, increased MAP to 47.1 ± 4.6 mmHg while cortical CBF increased to $65.5 \pm 6.9\%$ ($P < 0.05$ from the HS phase). In contrast to LR-RF, addition of DRP-RF improved mvCBF and tissue oxygenation, i.e., the number of perfused capillaries increased to 141.2 ± 6.8 per 0.075 mm^3 , and capillary flow velocity increased to 0.55 ± 0.04 leading to NADH autofluorescence reduction to $129.1 \pm 8.4\%$ (Fig. 1, $P < 0.05$ from the HS phase). Improved oxygen transport to tissue protected neurons from necrosis, as the number of dead neurons showed no significant increase: 62.3 ± 3.1 per 0.075 mm^3 (Fig. 2).

During the hospital phase in the LR-RF group, re-infusion of blood increased MAP to 70.2 ± 6.7 mmHg and cortical CBF to $76.8 \pm 8.4\%$. However, as in the pre-hospital phase, mvCBF and tissue oxygenation in the peri-contusion regions did not change significantly; however, there was a trend toward improvement (Fig. 1). The number of dead neurons increased to 177.3 ± 9.2 per 0.075 mm^3 of tissue by the end of this phase (Fig. 2). In the DRP-RF group, re-infusion of blood *during the hospital phase* increased MAP to 69.7 ± 5.9 mmHg and cortical CBF to $78.5 \pm 7.3\%$. mvCBF and tissue oxygenation further improved: the number of perfused capillaries increased to 162.3 ± 9.2 per 0.075 mm^3 , capillary flow velocity increased to 0.64 ± 0.05 , and NADH autofluorescence reduced to $121.8 \pm 9.1\%$ (Fig. 1, $P < 0.05$ from the LR-RF group). Improved oxygen transport to tissue protected neurons from necrosis, as the number of dead neurons was significantly less than in the LR-RF group: 78.2 ± 9.3 vs. 181.5 ± 10.2 per 0.075 mm^3 (Fig. 2c, $P < 0.05$). Anatomical T2 MRI at the end of the study revealed reduced lesion volumes in the DRP-RF compared to LR-RF ($P < 0.05$).

4 Discussion

Based on our data and previous studies [5,8], the mechanisms of restoring mvCBF by DRP-RF include increasing the arteriolar blood volume flow via the increase of flow velocity by reduction of flow separations and vortices at vessel bifurcations and decreasing pressure loss across the arterial network due to the viscoelastic properties of DRP. This leads to a rise in the pre-capillary blood pressure thus enhancing capillary perfusion, countering capillary stasis, increasing the density of functioning capillaries and the number of RBC passing through capillaries to improve tissue oxygenation. The decrease of cerebral hypoxia due to increased oxygen transport to tissue by restored mvCBF explains the neuroprotective effect

of DRP. Another beneficial property of DRP-RF is that it significantly reduced the amount of the fluid required to increase tissue perfusion, which is particularly essential for the traumatized brain. One of the main problems of current resuscitation fluids is the need for infusion of large volumes that can exacerbate brain edema [9] and cause hemodilution, thereby reducing blood oxygen carrying capacity that can further compromise oxygenation of the injured brain [10]. Adequate capillary perfusion, which can be restored by DRP-RF, is essential to maintain homeostasis, to remove metabolic waste products which, if allowed to accumulate, exert toxic effects, and to overcome deficits in oxygen delivery.

5 Conclusions

Rheological modulation of blood flow using advanced resuscitation fluid with DRP in nanomolar amounts effectively restores cerebral microcirculation, reduces hypoxia and protects neurons after TBI complicated by hemorrhagic shock compared to conventional volume expansion with lactated Ringer. In addition, DRP-RF requires infusion of a smaller volume to improve tissue perfusion and oxygen utilization which reduces brain edema formation due to hypervolemia, which often occurs with a standard fluid resuscitation.

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References

1. Manley G, Knudson MM, Morabito D et al. (2001) Hypotension, hypoxia, and head injury: frequency, duration, and consequences. *Arch Surg* 136 (10):1118–1123 [PubMed: 11585502]
2. Navarro JC, Pillai S, Cherian L et al. (2012) Histopathological and behavioral effects of immediate and delayed hemorrhagic shock after mild traumatic brain injury in rats. *J Neurotrauma* 29 (2):322–334 [PubMed: 22077317]
3. Bragin DE, Statom GL, Yonas H et al. (2014) Critical cerebral perfusion pressure at high intracranial pressure measured by induced cerebrovascular and intracranial pressure reactivity. *Crit Care Med* 42 (12):2582–2590 [PubMed: 25289933]
4. Bragin DE, Bush RC, Muller WS et al. (2011) High intracranial pressure effects on cerebral cortical microvascular flow in rats. *J Neurotrauma* 28 (5):775–785 [PubMed: 21395499]
5. Bragin DE, Kameneva MV, Bragina OA et al. (2017) Rheological effects of drag-reducing polymers improve cerebral blood flow and oxygenation after traumatic brain injury in rats. *J Cereb Blood Flow Metab* 37 (3):762–775 [PubMed: 28155574]
6. Kameneva MV, Wu ZJ, Uraysh A et al. (2004) Blood soluble drag-reducing polymers prevent lethality from hemorrhagic shock in acute animal experiments. *Biorheology* 41 (1):53–64 [PubMed: 14967890]
7. Fumagalli S, Coles JA, Ejlerskov P et al. (2011) In vivo real-time multiphoton imaging of T lymphocytes in the mouse brain after experimental stroke. *Stroke* 42 (5):1429–1436 [PubMed: 21441145]
8. Kameneva MV (2012) Microrheological effects of drag-reducing polymers in vitro and in vivo. *Int J Eng Sci* 59:168–183
9. Falk JL (1995) Fluid resuscitation in brain-injured patients. *Crit Care Med* 23 (1):4–6 [PubMed: 8001385]
10. Lee EJ, Hung YC, Lee MY (1999) Anemic hypoxia in moderate intracerebral hemorrhage: the alterations of cerebral hemodynamics and brain metabolism. *J Neurol Sci* 164 (2):117–123 [PubMed: 10402021]

Fig. 1.

Resuscitation with DRP-RF improves cerebral microvascular perfusion and tissue oxygenation impaired by TBI/HS, as shown by: a) increased number of perfused capillaries; b) increased capillary flow velocity; c) increased tissue oxygenation (NADH decrease).

Mean $\pm$ SEM, N=10 rats per group, *P < 0.05 from the LR-RF group.

Fig. 2.

Resuscitation with DRP-RF is neuroprotective: a) 2PLSM image of a rat cortex at baseline without dead neurons; b) Propidium Iodide stains neurons with damaged membranes reflecting necrosis of neurons after TBI/HS; c) DRP-RF protects neurons from necrosis (*= $P < 0.05$). Mean $\pm$ SEM, N=10 rats per group, * $P < 0.05$ from the LR-RF group.