Improvement of Impaired Cerebral Microcirculation Using Rheological Modulation by Drag-Reducing Polymers

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Abstract

Nanomolar intravascular concentrations of drag-reducing polymers (DRP) have been shown to improve hemodynamics and survival in animal models of ischemic myocardium and limb, but the effects of DRP on the cerebral microcirculation have not yet been studied. We recently demonstrated that DRP enhance microvascular flow in normal rat brain and hypothesized that it would restore impaired microvascular perfusion and improve outcomes after focal ischemia and traumatic brain injury (TBI). We studied the effects of DRP (high molecular weight polyethylene oxide, 4,000 kDa, *i.v.* at 2 μg/mL of blood) on microcirculation of the rat brain: 1) after permanent middle cerebral artery occlusion (pMCAO); and 2) after TBI induced by fluid percussion. Using *in-vivo* two-photon laser scanning microscopy (2PLSM) over the parietal cortex of anesthetized rats we showed that both pMCAO and TBI resulted in progressive decrease in microvascular circulation, leading to tissue hypoxia (NADH increase) and increased blood brain barrier (BBB) degradation. DRP, injected post insult, increased blood volume flow in arterioles and red blood cell (RBC) flow velocity in capillaries mitigating capillary stasis, tissue hypoxia and BBB degradation, which improved neuronal survival (Fluoro-Jade B, 24 hours) and neurologic outcome (Rotarod, 1 week). Improved microvascular perfusion by DRP may be effective in the treatment of ischemic stroke and TBI.

1 Introduction

Although ischemic stroke and traumatic brain injury (TBI) arise from very different initial insults, the mechanisms involved in the injury process have many similarities [1]. Among them is a reduction in cerebral blood flow (CBF) leading to microvascular circulation impairment and deprivation of oxygen and glucose delivery to tissue.

Currently, there are no approved therapies targeting impaired CBF after TBI and only one targeting impaired CBF after ischemic stroke, but that one is limited by a short treatment window.

Rheological modulation of the blood circulation by minute quantities of drag reducing polymers (DRP) was demonstrated to improve circulation and survival in animal models of hemorrhagic shock, ischemic myocardium and limbs. However, except for one qualitative study [2], the effects of DRP on the impaired cerebral circulation have not yet been studied.

We recently showed that DRP enhanced microvascular flow and tissue oxygenation in a healthy rat brain. Here, we examine the acute effects of DRP on impaired cerebral microcirculation and oxygen delivery after TBI induced by fluid percussion, and ischemic stroke induced by permanent middle cerebral artery occlusion (pMCAO), and the translation of these effects into long-term neurologic outcome and function.

2 Methods

2.1 Study Paradigm

Most of the procedures used in these studies were previously described [3, 4]. The fluid percussion was used as a model of TBI and was induced by 1.5 ATA 50 ms pulse from a custom-built Pneumatic Impactor connected to the brain through a pressure transducer filled with artificial cerebrospinal fluid [5]. The suture pMCAO was used as a model of ischemic injury [6].

The acute effects of DRP on microvascular blood flow velocity, tissue oxygenation (NADH) and blood brain barrier (BBB) permeability were measured on laboratory acclimated, male, Sprague-Dawley rats weighing 250–300 grams by two-photon laser scanning microscopy (2PLSM) under 2% isoflurane/69%nitrous oxide/28% oxygen anesthesia via a cranial window over the left parietal cortex. After baseline imaging, TBI or pMCAO was induced and, after post-insult imaging and DRP (2 μg/ml in blood) or saline *i.v.* injection, the consequent imaging was followed during 4 hours. 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 controlled at $38\pm0.5°C$. Arterial blood gases, electrolytes, hematocrit and pH were measured hourly.

Neurodegeneration was examined by Fluoro-Jade B staining at 24 hours after insult [7]. Coordination and motor deficits were evaluated by Rotarod at one week after insults [8]. Time to dismount with increasing speed of rotation was used as a measure of deficit.

2.2 Two-Photon Laser Scanning Microscopy

Fluorescent serum (tetramethylrhodamine isothiocyanate (TAMRA) dextran, 500 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 center wavelength. Band-pass-filtered epifluorescence (560–660 nm for TAMRA and 425–475 nm for NADH) was collected by photomultiplier tubes of the Prairie View Ultima system. Images (512 X 512 pixels, 0.15 um/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 3–50 μm diameter up to 500 μm below the surface of the parietal cortex as previously described [3, 4]. Tissue hypoxia was assessed by measurement of NADH autofluorescence and BBB permeability by TAMRA transcapillary extravasation. 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).

Statistical analyses were done by Student's t-test or Kolgomorov-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. Statistical significance level was set at $P < 0.05$.

3 Results

3.1 Acute effects on cerebral blood flow and metabolism

Both TBI and pMCAO progressively decreased microvascular circulation with development of tissue hypoxia and BBB damage in peri-injury or penumbra zones of the rat brain, respectively. DRP injection significantly increased arteriolar blood volume flow in both, TBI (from $71.3 \pm 10.5\%$ to $84.3 \pm 9.4\%$) and pMCAO (from $67.3 \pm 11.3\%$ to $81.8 \pm 10.2\%$) that was not observed in saline-treated animals (Fig. 1a, $p < 0.05$). The increase in arteriolar blood volume flow restored circulation in collapsed capillaries by $24 \pm 6.7\%$ and $31.0 \pm 7.1\%$ compared to saline after TBI and pMCAO, respectively (Fig. 1b, $p < 0.05$). The improved capillary perfusion increased tissue oxygenation reflected by a decrease of NADH autofluorescence by $18 \pm 4.6\%$ and $17.0 \pm 6.5\%$ comparing saline after TBI and pMCAO, respectively (Fig. 1c, $p < 0.05$). DRP also reduced BBB damage in microvessels, as quantified by measurement of perivascular tissue fluorescence reflecting the rate of TAMRA dextran extravasation. The fluorescence ratios ($F/Fo_{[pre-injury]}$) were 8.7 ± 0.8 vs. 7.1 ± 0.9 for TBI saline vs. DRP and 8.3 ± 0.9 vs. 7.2 ± 0.6 for pMCAO saline vs. DRP, respectively (Fig. 1d, $p < 0.05$).

3.2 Long-term outcome

The microvascular flow and tissue oxygenation improved by DRP reduced neurodegeneration by $37.6 \pm 11.2\%$ (TBI) and $44.2 \pm 12.6\%$ (pMCAO) compared to saline as measured 24 hours post-insult by Fluoro-Jade B staining (Fig. 2a, $p<0.05$). Evaluation of neurological motor deficits by Rotarod tests at one week after insults revealed that DRP-treated rats performed significantly better than saline-treated animals. Rotarod latency times were $75.6 \pm 16.0\%$ vs. $47.3 \pm 15.2\%$, for TBI-DRP vs. TBI-saline rats and $78.2 \pm 19.0\%$ vs. $51.4 \pm 17.3\%$, for pMCAO-DRP vs. pMCAO-saline from a baseline, respectively (Fig. 2b, $p<0.05$).

4 Discussion

After TBI or ischemic stroke, DRP improve impaired cerebral microvascular perfusion thereby reducing tissue hypoxia and BBB degradation and protecting neurons from death, which translates into improved neurologic outcome. Based on our data and previous in-vitro [9, 10] and in-vivo [11] studies, we could speculate that the mechanisms of increasing the arteriolar blood volume flow include an increase of flow velocity by reduction of flow separations and vortices at vessel bifurcations and increasing a pressure gradient across the arterial vessel network due to the viscoelastic properties of DRP. This leads to a rise in the

pre-capillary blood pressure thus enhancing capillary perfusion and countering capillary stasis.

DRP also reduce the near-wall cell-free layer increasing wall shear stress and decreasing plasma skimming at vessel bifurcations (i.e., lowering hematocrit in daughter branches of microvessels), described in vitro [12, 13] and in vivo [14] which could explain protection of the BBB. Recent studies reported that increased wall shear stress restrains expression of proinflammatory cytokines that initiate a signaling cascade leading to activation of BBB damage [15, 16]

Due to the absence of effective therapies for impaired cerebral microcirculation leading to tissue hypoxia, despite the numerous pharmacological treatments successfully tested in animals that failed clinically, the use of DRP is a promising approach for the treatment of TBI, ischemic stroke and other low CBF pathologic conditions.

Acknowledgments

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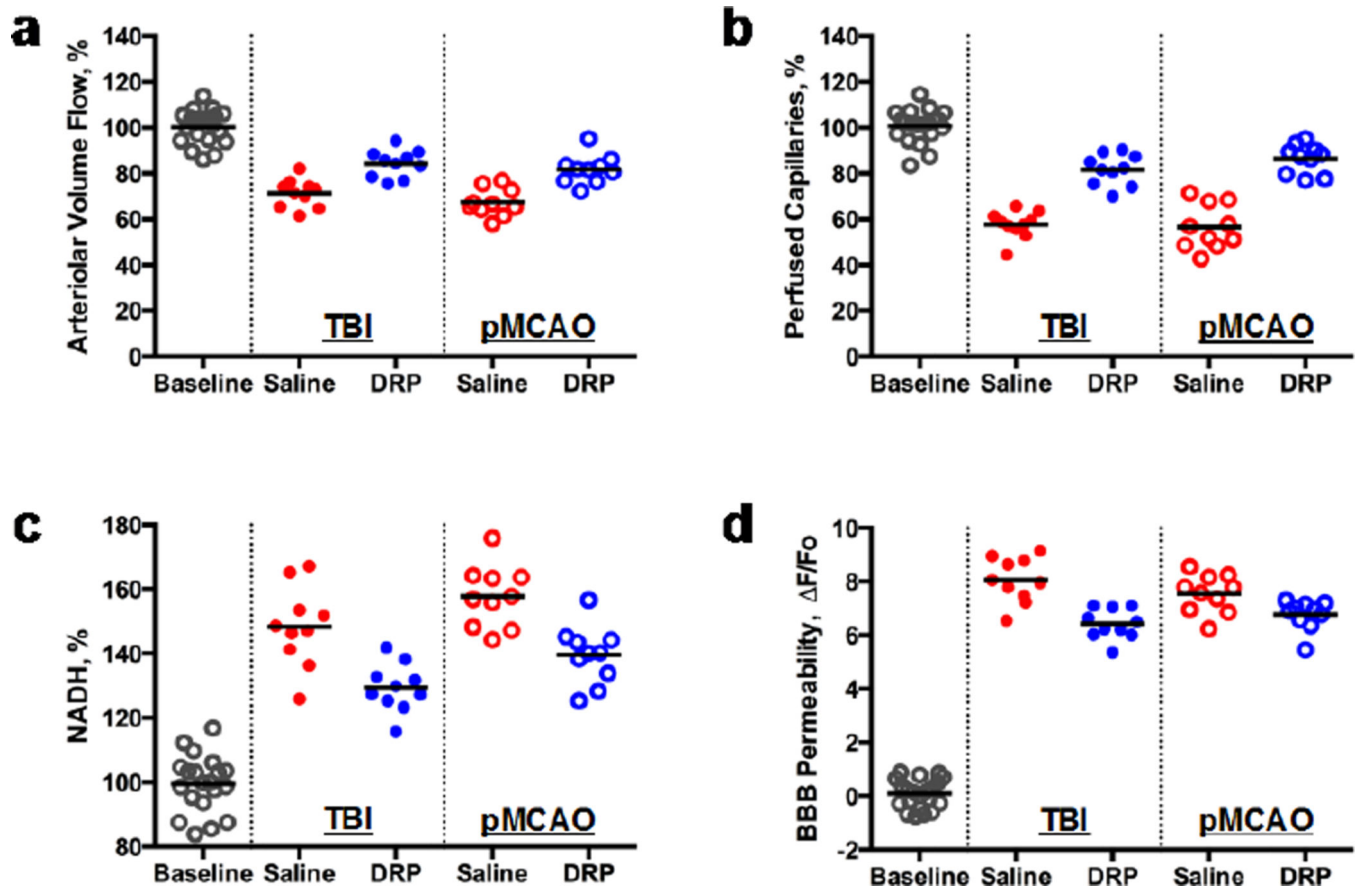
Fig. 1.
Intravenous injection of DRP in acute period of TBI or pMCAO: (a) increases arteriolar volume flow; (b) increases number of perfused capillaries; (c) improves tissue oxygenation (NADH); and (d) attenuates BBB damage (N=10 rats per group).
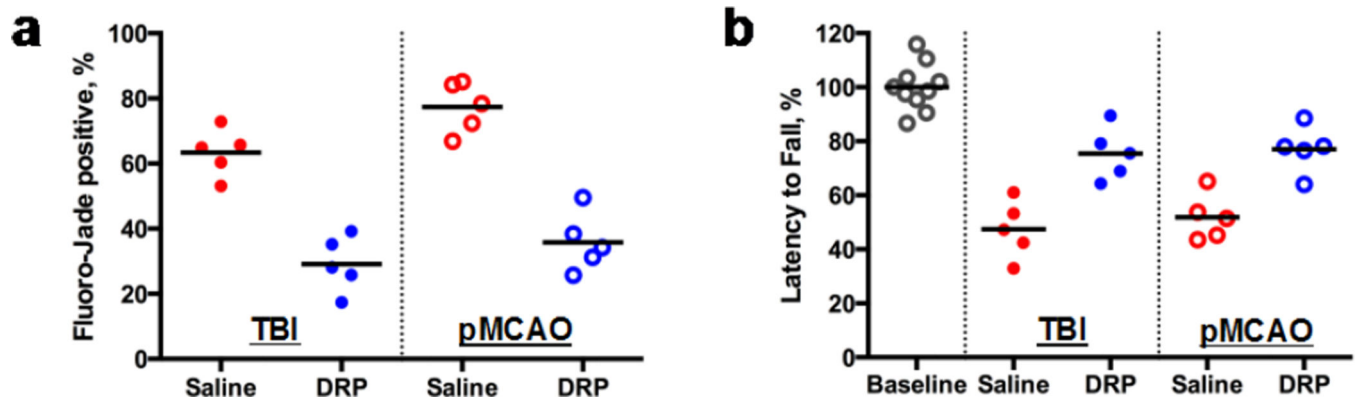
Fig. 2.
(a) Intravenous injection of DRP reduces neurodegeneration as measured 24 hours post-insult by Fluoro-Jade B staining and (b) improves neurologic outcome as measured by Rotarod test for motor and coordination deficits 1 week after TBI or pMCAO (N=5 rats per group).