Drag-Reducing Polymer Enhances Microvascular Perfusion in the Traumatized Brain with Intracranial Hypertension

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Abstract Current treatments for traumatic brain injury (TBI) have not focused on improving microvascular perfusion. Drag-reducing polymers (DRP), linear, long-chain, blood-soluble, nontoxic macromolecules, may offer a new approach to improving cerebral perfusion by primary alteration of the fluid dynamic properties of blood. Nanomolar concentrations of DRP have been shown to improve hemodynamics in animal models of ischemic myocardium and ischemic limb, but have not yet been studied in the brain. We recently demonstrated that DRP improved microvascular perfusion and tissue oxygenation in a normal rat brain. We hypothesized that DRP could restore microvascular perfusion in hypertensive brain after TBI. Using in vivo two-photon laser scanning microscopy we examined the effect of DRP on microvascular blood flow and tissue oxygenation in hypertensive rat brains with and without TBI. DRP enhanced and restored capillary flow, decreased microvascular shunt flow, and, as a result, reduced tissue hypoxia in both nontraumatized and traumatized rat brains at high intracranial pressure. Our study suggests that DRP could constitute an effective treatment for improving microvascular flow in brain ischemia caused by high intracranial pressure after TBI.

Keywords Drag-reducing polymer • Polyethylene oxide (PEO) • Traumatic brain injury • Intracranial pressure • Cerebral blood flow • Capillaries • Microvascular shunts • NADH • Hypoxia • Ischemia • Rats

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Introduction

Ischemia is a secondary injury that frequently occurs after traumatic brain injury (TBI). Oxygen and nutrient deprivation ultimately leads to permanent cell death. Currently, none of the treatments for TBI has focused on restoring or improving microvascular perfusion after TBI. Drag-reducing polymers (DRP), linear, long-chain, blood-soluble, nontoxic macromolecules, may offer a new approach to improving cerebral perfusion by primary alteration of the fluid dynamic properties of blood. DRP have been shown to improve hemodynamics and survival in animal models of ischemic myocardium [1–3], ischemic limb [4], and hemorrhagic shock [5, 6]. However, despite their promising therapeutic potential, DRP have not yet been studied in the brain. In a single observational, qualitative study of rabbits, intravenous injection of DRP restored brain circulation after global ischemia caused by permanent occlusion of the carotid and vertebral arteries [7].

The increased intracranial pressure (ICP) after TBI, among other detrimental consequences, restricts blood supply to the tissue, that is, causes ischemia. In previous studies we showed that high ICP compromised capillary flow, leading to the transition of the blood flow to nonnutritive microvascular shunts (MVSs) in both nontraumatized [8] and traumatized [9] brains. This transition was accompanied by tissue hypoxia, brain edema, and blood–brain barrier damage [8, 9].

In this study we examined the effects of intravenous DRP on the nontraumatized and traumatized rat brain at high ICP by in vivo two-photon laser scanning microscopy.

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Materials and Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of New Mexico Health Sciences Center and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Paradigm

Two models were used in this study:

1. Intracranial hypertension, where ICP was increased from a normal 10 to 40 mmHg by the vertical positioning of an artificial cerebrospinal fluid (ACSF) reservoir connected to the cisterna magna
2. TBI resulting in an increase in ICP by fluid percussion using a custom-built pneumatic impactor connected to a transducer filled with ACSF and glued over a craniotomy above the left parietal cortex for transmission pressure onto the brain (1.5 ATA, 100-ms pulse duration)

Using in vivo two-photon laser scanning microscopy through a cranial window over the parietal cortex (the peri-contusion area in TBI), we measured microvascular red blood cell flow velocity visualized by serum labeled with tetramethylrhodamine dextran and nicotinamide adenine dinucleotide (NADH) autofluorescence for tissue oxygenation. Arterial pressure; blood gases, electrolytes, hematocrit and pH; rectal and cranial temperatures were monitored and maintained within normal values throughout the studies. All measurements were carried out at baseline and after ICP increase or after trauma induction time points. DRP (1 μg/ml blood) was injected i.v. after an increase in ICP (5 rats) or after TBI (5 rats). For TBI control, an additional 5 animals were injected with vehicle (normal saline).

Surgery

Most of the procedures used in this study have been previously described [8, 10]. Briefly, acclimated Sprague–Dawley male rats (Harlan Laboratories, Indianapolis, IN, USA), weighing between 300 and 350 g, were intubated and mechanically ventilated on 2 % isoflurane/30 % oxygen/70 % nitrous oxide. Rectal and temporal muscle probes were inserted. Femoral venous and arterial catheters were inserted for injections, arterial pressure monitoring, and blood sampling. A catheter was inserted into the cisterna magna for ICP monitoring and manipulation. For imaging and TBI, a craniotomy 5 mm in diameter was made over the left parietal cortex, filled with 2 % agarose/saline, and sealed with a cover glass.

Microscopy

An Olympus BX51WI upright microscope and a water-immersion LUMPlan FL/IR 20×/0.50 W objective were used. Excitation (740 nm) was provided by a Prairie View Ultima multiphoton 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). Blood plasma was labeled by i.v. injection of tetramethylrhodamine isothiocyanate dextran (155 kDa) in physiological saline (5 % wt/vol). All microvessels in an imaging volume (500 × 500 × 300 μm) were scanned at each study point, measuring the diameter and blood flow velocity in each vessel (3–20 μm Ø). Tetramethylrhodamine fluorescence was band pass filtered at 560–600 nm and NADH autofluorescence at 425–475 nm. Imaging data processing and analysis were carried out using the Fiji image processing package [11].

Statistical Analyses

Statistical analyses were carried out using Student's *t* test or the 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. The statistical significance level was set at $P<0.05$. Data are presented as mean ± SEM.

Results

Intracranial Hypertension

At normal ICP of 10 mmHg, microvascular RBC flow velocity in microvessels ranged from 0.12 to 4.05 mm/s with normal frequency distribution, as was measured in an imaging volume of (500 × 500 × 300 μm) by line scans in each microvessel ranging from 3 to 20 μm in diameter (Fig. 1a). An ICP increase to 40 mmHg caused redistribution of microvascular flow; capillary flow (diameter of 3–8 μm and velocities <1 mm/s) was compromised, which led to the transition of flow to the MVS (diameter 8–20 μm and velocities >1 mm/s) as reflected by the

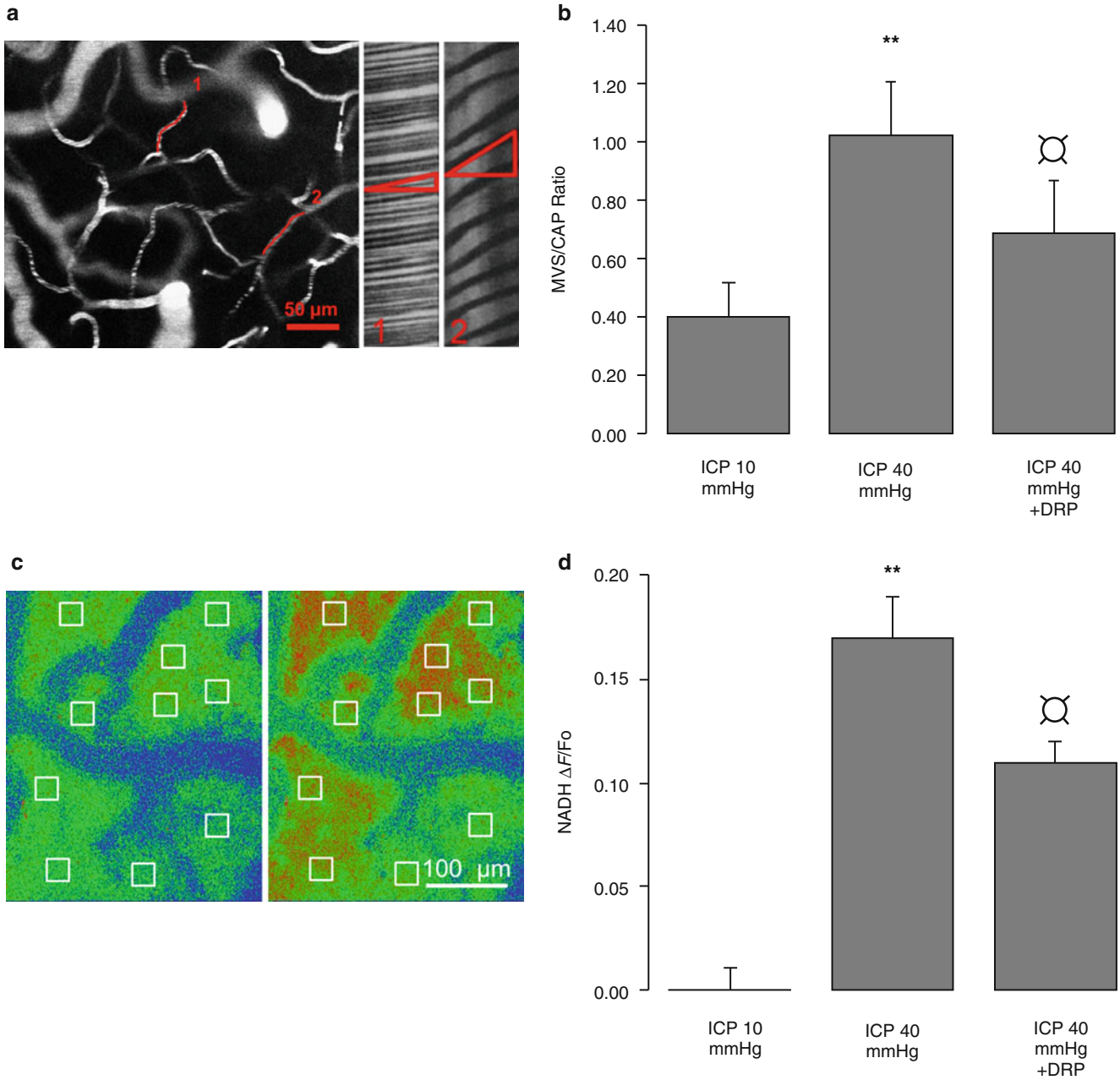

Fig. 1 (**a**) *Left*: a representative in vivo two-photon laser scanning microscopy micrograph showing a region from which microvascular flow was recorded. *Right*: line scan data of red blood cell flow velocities in the two microvessels shown on the *left*. A line scan through a microvessel leads to a sequence of alternating *bright* and *dark* pixels corresponding to labeled plasma and unlabeled red blood cells (RBC). This results in diagonal bands on a space–time image, as illustrated. The slope of the stripes inversely reflects RBC velocity; the second microvessel has a lower RBC flow velocity than the first. (**b**) Changes in microvascular shunt/capillary flow (MVS/CAP) ratio showing that drag-reducing polymers (DRP) attenuated MVS flow, which is elevated at a high intracranial pressure (ICP) of 40 mmHg. (**c**) Representative in vivo two-photon laser scanning microscopy micrographs with regions of interest (ROI) of nicotinamide adenine dinucleotide (NADH) autofluorescence show an increase in tissue hypoxia after ICP elevation to 40 mmHg (*right*) compared with baseline ICP of 10 mmHg (*left*). (**d**) Graph shows that DRP reduced tissue hypoxia caused by ICP elevation to 40 mmHg, as reflected by an increase in NADH. Data were presented as a ratio $\Delta F/Fo$, where Fo is NADH at ICP = 10 mmHg. All data are presented as mean ± SEM, $n = 5$, $^{**}P < 0.01$ compared with a baseline ICP of 10 mmHg, $^{¤}P < 0.05$ compared with an ICP of 40 mmHg

increase in the capillary/MVS ratio (CAP/MVS) to 1.02 ± 0.19 compared from a baseline MVS/CAP ratio of 0.42 ± 0.12 at an ICP of 10 mmHg (Fig. 1b, $P < 0.01$). DRP enhanced capillary flow and reduced MVS flow, as indicated by the decrease in the MVS/CAP ratio to 0.69 ± 0.18 (Fig. 1b, $P < 0.05$) compared with ICP of 40 mmHg before DRP injection.

The increase in ICP to 40 mmHg caused a significant increase in NADH autofluorescence ($\Delta F/Fo_{[ICP=10\ mmHg]} = 0.16 \pm 0.02$, Fig. 1d, $P < 0.01$). NADH is a sensitive indicator of

tissue hypoxia; reduced (NADH) is fluorescent, whereas the oxidized form (NAD^+) is not; therefore, increased fluorescence reflects the accumulation of NADH, which occurs because of reduced tissue oxygenation (Fig. 1c) [12, 13]. DRP decreased NADH autofluorescence, indicating improved tissue oxygenation related to enhanced microvascular perfusion ($\Delta F/Fo_{[ICP=10\ mmHg]} = 0.11 \pm 0.01$, Fig. 1d, $P < 0.05$) compared with an ICP of 40 mmHg before injection.

Traumatic Brain Injury with Intracranial Hypertension

Fluid percussion injury in the saline-treated group resulted in a sustained increase in ICP to 30.8 ± 4.7 mmHg from the pre-injury level of 10.3 ± 3.6 mmHg ($n = 5$, $P < 0.01$). In the DRP-treated group, the ICP only increased to 26.9 ± 6.5 mmHg from the pre-injury level 10.5 ± 4.1 mmHg ($n = 5$, $P < 0.05$); however, the difference between saline- and DRP-treated groups was not statistically significant ($P = 0.18$). Arterial pressure in both groups was unaltered.

In a control group, the rise in ICP was associated with an increase in the MVS/CAP ratio from 0.43 ± 0.09 before injury to 1.39 ± 0.23 after injury (Fig. 2a, $P < 0.01$). In DRP-treated group, the MVS/CAP increased from 0.42 ± 0.08 before injury to 0.85 ± 0.25 after injury (P < 0.05), and was significantly lower than in the control group (Fig. 2b, $P < 0.05$). Therefore, DRP attenuated pathological MVS flow and enhanced capillary flow.

Traumatic brain injury compromised capillary perfusion. In the peri-contusion area of a saline-treated brain the percentage of perfused capillaries decreased to 47.3 ± 14.4 % compared with a baseline (Fig. 2b, $P < 0.01$). In DRP-treated brain, the amount of capillaries with collapsed perfusion was reduced to only 72.1 ± 15.84 % compared with a baseline (Fig. 2b, $P < 0.05$). This was significantly less than in the control saline-treated group ($P < 0.05$).

Posttraumatic microvascular flow impairment in the saline-treated group led to tissue hypoxia, reflected by NADH accumulation ($\Delta F/Fo_{[pre\text{-}injury]} = 0.59 \pm 0.09$, Fig. 1c, $P < 0.01$) compared with a baseline. Improved microvascular flow in the DRP-treated group mitigated tissue hypoxia; NADH autofluorescence only increased to 0.24 ± 0.05 (Fig. 1c, $P < 0.05$ compared with a baseline and $P < 0.05$ compared with the saline-treated group).

Discussion

The intravascular mechanisms of DRP action are not completely understood. These long, molecules of DRP, dissolved in blood plasma, are thought to provide a "liquid scaffold," reducing pressure loss in small arteries and arterioles by organizing blood flow and suppressing flow separations and vortices at vascular branch points [5, 14–18]. In addition, DRP reduces "plasma skimming" at vessel bifurcations, which increases red blood cell (RBC) flow in the capillaries [14, 16]. The increase in the precapillary pressure promoting an increase in the density of functioning capillaries and the elimination of capillary stasis caused by ischemia or other

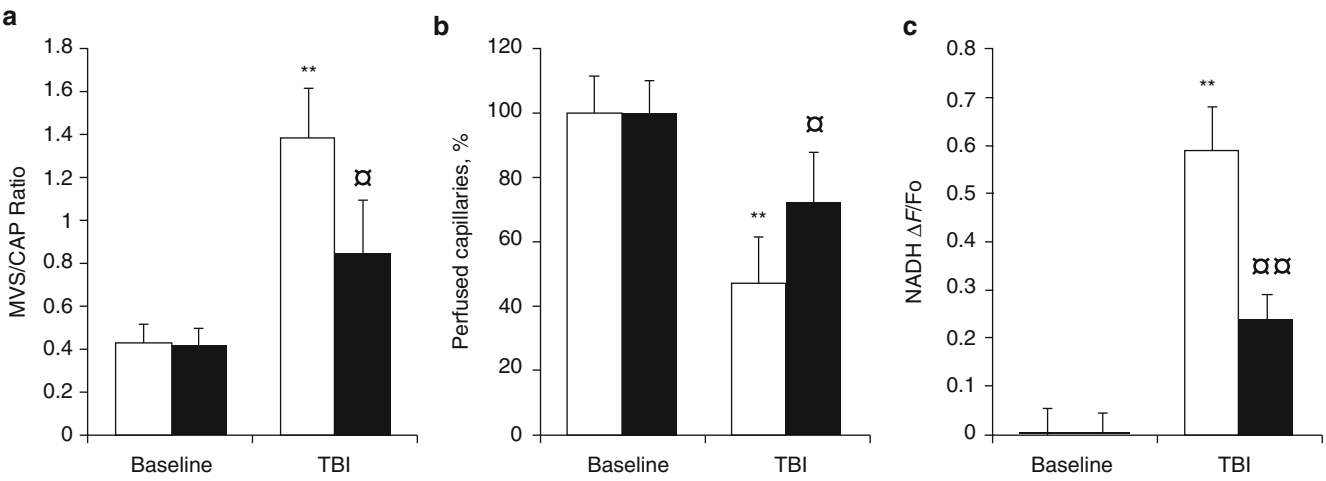

Fig. 2 (**a**) Bar graph showing that the posttraumatic increase in microvascular (MVS) flow was less in the DRP group than in saline controls, as reflected by the MVS/capillary (CAP) ratio. ■ DRP-treated group, □ saline-treated control group. (**b**) Bar graph showing that after TBI fewer capillaries collapsed in the DRP-treated group than in the saline control group. (**c**) Bar graph showing that DRP-treated animals had less cortical tissue hypoxia than saline control animals, as reflected by NADH autofluorescence. Data are presented as $\Delta F/Fo$, where Fo is pre-TBI baseline. All data are presented as mean ± SEM, $n = 5$ per group, $^{**}P < 0.01$ compared with baseline ICP of 10 mmHg, ¤$P < 0.05$ compared with an ICP of 40 mmHg

pathological conditions [14]. The net effect is improved microcirculation and increased red blood cell (RBC) traffic in the microvessels [16–18].

We previously reported that in a healthy rat brain DRP increased near-wall blood flow velocity in arterioles and reduced plasma skimming at bifurcations, leading to increased blood volume perfused through the vessel resulting in an increase in the number of RBCs entering the capillaries [19]. This led to enhanced capillary perfusion and increased tissue oxygenation. In this study we showed that DRP reduced pathologically elevated nonnutritive microvascular shunt flow and partially restored perfusion in collapsed capillaries, resulting in reduced tissue hypoxia in nontraumatized and traumatized rat brains at high ICP. The effect of a decrease in ICP by DRP is not clear, but could be connected to the decrease in pathological MVS flow and enhancement of capillary perfusion. In summary, our studies demonstrated that DRP could provide a novel hemorheological approach to the treatment of brain ischemia caused by blood flow restriction in traumatized brain, based on primary modulation of the flow properties of blood. The long-term effects of DRP treatment on neurological outcome after TBI are currently under investigation.

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Conflict of Interest Statement We declare that we have no conflict of interest.

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