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Induced Dynamic Intracranial Pressure Reactivity (iPRx) and Cerebrovascular Reactivity (iCVRx) Assessment of Cerebrovascular Autoregulation after Traumatic Brain Injury with High Intracranial Pressure in Rats.

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Abstract

Objective: In previous work we have shown that high intracranial pressure (ICP) rat brain induces a transition from capillary (CAP) to pathological microvascular shunt flow (MVS) resulting in brain hypoxia, edema and blood brain barrier (BBB) damage. In a normal brain this transition was correlated with loss of cerebral blood flow (CBF) autoregulation undetected by static autoregulatory curves but identified using *induced* dynamic ICP (iPRx) and cerebrovascular (iCVRx) reactivity. We hypothesized that loss of autoregulation correlated with MVS flow will be identified by iPRx and iCVRx in traumatic brain injury (TBI) with elevated ICP.

Methods: TBI was induced by lateral fluid percussion (LFP) using a gas-driven device in rats. Using *in vivo* two-photon laser scanning microscopy, cortical microcirculation, tissue oxygenation (NADH autofluorescence), and blood brain barrier (BBB) permeability (fluorescein dye extravasation) were measured before and for 4 hours after TBI. Doppler cortical flux, temperature, ICP and mean arterial pressure (MAP), blood gases and electrolytes were monitored. Every 30 minutes, a transient 10-mmHg rise in MAP was induced by i.v. bolus of dopamine. $iPRx = \frac{ICP}{MAP}$ and $iCVRx = \frac{CBF}{MAP}$.

Results: We demonstrated that iPRx and iCVRx correctly identified more severe loss of CBF autoregulation correlated with transition of blood flow to MVS after TBI with high ICP compared to TBI without an increase in ICP.

Conclusions: In TBI with high ICP, high velocity MVS flow is responsible for loss of cerebral autoregulation identified by iPRx and iCVRx.

Keywords

Traumatic brain injury; Cerebral blood flow autoregulation; Microvascular shunts; Intracranial pressure; Induced cerebrovascular reactivity; Induced intracranial pressure reactivity; Rats

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

1 Introduction

Cerebrovascular autoregulation is the ability of the brain to maintain cerebral blood flow (CBF) despite changes in cerebral perfusion pressure (CPP), a difference between MAP and ICP. The critical CPP is the pressure at which CBF begins to fall after maximum cerebrovascular dilation has occurred and historically determined at ~50 mmHg by decreasing arterial pressure to lower CPP while tracking changes in CBF [1]. However, clinically CPP often decreases due to an increase in intracranial pressure (ICP) as in traumatic brain injury (TBI).

To test that, several animal studies used increased ICP to decrease CPP instead of decreasing arterial pressure and reported loss of autoregulation at ~30 mmHg [2-5]. The reason for an “apparently” better preserved autoregulation at a lower CPP was unclear until we showed that in a normal rat brain high ICP-induced a transition of capillary blood flow to high velocity, non-nutritive microvascular shunt (MVS) flow that was associated with brain hypoxia, edema and blood brain barrier (BBB) damage [6-7]. This transition was correlated with loss of CBF autoregulation undetected by static autoregulatory curves but identified by *induced* dynamic ICP (iPRx) and cerebrovascular (iCVRx) reactivity [8]. More recently we proved existence of MVS flow in cerebral microcirculation after traumatic brain injury (TBI) with high ICP [9].

In this study we determined whether iPRx and iCVRx accurately identifies loss of CBF autoregulation correlated with the transition of blood flow to MVS in TBI with high ICP.

2 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. The procedures used in this study have been described in our previous publications [6-9].

Experimental Paradigm

Two groups of 10 rats each were used in this study:

- I. TBI resulting in a permanent increase in ICP=30 mmHg;
- II. TBI without ICP increase (ICP=10 mmHg).

We used *in-vivo* two-photon laser scanning microscopy (2PLSM) through a cranial window over the peri-contusion area of parietal cortex to measure microvascular red blood cell flow velocity, visualized by serum labeled with tetramethylrho-damine dextran (TMR), and tissue oxygenation, reflected by nicotinamide adenine dinucleotide (NADH) autofluorescence, for 4 hours after TBI. Blood brain barrier (BBB) permeability was measured by TMR extravasation. Doppler cortical flux; MAP and ICP; blood gases, electrolytes, hematocrit and pH; rectal and cranial temperatures were monitored throughout the study. Every 30 minutes, a transient 10-mmHg rise in MAP was induced by i.v. bolus of dopamine. $iPRx = \frac{ICP}{MAP}$ and $iCVRx = \frac{CBF}{MAP}$.

Surgery

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. TBI was induced by fluid percussion injury (FPI) using a gas-driven device in rats resulting in: 1) No ICP increase (1.5 ATA, 50 ms pulse duration); and 2) Persistent ICP increase for up to six hours (1.5 ATA, 150 ms pulse duration).

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. tetramethylrhodamine isothiocyanate dextran (155 kDa) in physiological saline (5 % wt/ vol). All microvessels in an imaging volume ($500 \times 500 \times 300 \mu\text{m}$) were scanned at each study point, measuring the diameter and blood flow velocity in each vessel ($3\text{--}20 \mu\text{m}$ 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 NIH ImageJ.

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 $\pm$ SEM.

3 Results

As in our previous report, FPI in group I resulted in a sustained increase in ICP to 30.9 ± 4.4 mmHg from the pre-injury level of 10.1 ± 2.7 mmHg ($P < 0.01$); in group II ICP was not changed (10.8 ± 3.6 mmHg). Arterial pressure in both groups was unaltered.

In group I the rise in ICP was associated with an increase in the MVS/CAP ratio from 0.41 ± 0.07 at baseline to 1.41 ± 0.32 at four hours after TBI (Table 1, $P < 0.01$). The percentage of perfused capillaries decreased to 46.9 ± 16.5 % compared to baseline ($P < 0.001$) and microvascular CBF (mCBF) fell from 0.74 ± 0.09 at baseline to 0.53 ± 0.94 (Table 1, $P < 0.05$ from baseline). Impairment of microcirculation in group I led to tissue hypoxia, reflected by NADH accumulation ($172.8 \pm 9.6\%$, $P < 0.01$) and increased BBB permeability reflected by an increase in perivascular tissue fluorescence ($F/F_{0[\text{pre-injury}]} = 12.43 \pm 1.02$, Table 1, $P < 0.01$), both compared to a baseline.

In group II without an ICP increase, microvascular CBF fell to 0.48 ± 0.08 mm/s (Table 1, $P < 0.05$) and the percentage of perfused capillaries decreased to 72.3 ± 14.6 % ($P < 0.05$) compared to baseline. Tissue hypoxia and BBB damage were less prominent than in group I with high ICP (Table 1, $P < 0.05$ for both) with (NADH = 154.5 ± 8.3 %, $P < 0.05$) and increased tissue fluorescence ($F/Fo_{[pre-injury]} = 9.15 \pm 0.98$, $np < 0.05$) from baseline.

Evaluation of dynamic cerebrovascular reactivity in TBI group I with high ICP showed impaired CBF autoregulation as iCVRx increased to 0.61 ± 0.03 from -0.02 ± 0.05 , respectively ($P < 0.01$ from baseline); in group II iCVRx increased only to 0.37 ± 0.02 (Table 1, $P < 0.05$ from baseline and group I).

Dynamic ICP reactivity also revealed higher impairment in group I with high ICP than in group II without ICP increase: iPRx values were 0.35 ± 0.02 vs. 0.26 ± 0.02 , $P < 0.02$ compared to -0.02 ± 0.07 at baseline, respectively (Table 1, $P < 0.05$ for both).

4 Discussion

Our results show that despite apparently similar CBF reduction as measured by Doppler, CBF autoregulation was more impaired in the TBI group with increased ICP suggesting that high ICP-induced a transition from capillary to MVS flow involved in the loss of CBF autoregulation in the injured brain. In fact, 2PLSM showed that capillary flow in TBI with high ICP was more damaged than in TBI without ICP rise and preserved CBF in group I was mimicked by high-velocity non-nutritive MVS flow. Passive CBF autoregulation curves fails because they passively decreases CPP and measures the decrease in CBF based upon the overall average flow rate through the microvasculature flow compartments. In the induced dynamic method, an arterial transient into the cerebral microvasculature differentially impacts capillaries and MVS and the response in ICP and CBF depends upon the degree of MVS or the MVS/CAP ratio, which we suggest correlates with the degree of loss of autoregulation as a fraction of capillary flow. As the MVS/CAP ratio increases so does the proportion of tissue with loss of autoregulation as reflected by the increase in iPRx and iCVRx (Figure 1).

5 Conclusion

Dynamic iPRx and iCVRx accurately identified impaired CBF autoregulation after TBI with and without an increase in ICP.

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Figure 1.

Correlation between MVS/CAP flow ratio and iCVRx (a) and iPRx (b) plotted for every hour after TBI with high ICP. Open circles – baseline. Red dotted line – linear fit.

Table 1.Monitored variables (mean $\pm$ SEM)

Groups	mCBF, mm/s	MVS/CAP ratio	Perfused Capillaries, %	NADH, %	BBB, F/F ₀	iCVR _x	iPR _x
Baseline	0.74 $\pm$ 0.09	0.41 $\pm$ 0.07	100	100	0	-0.02 $\pm$ 0.05	-0.02 $\pm$ 0.07
TBI - I	0.53 $\pm$ 0.94 **	1.41 $\pm$ 0.32 **	46.9 $\pm$ 16.5 ***	172.8 $\pm$ 9.6 **	12.43 $\pm$ 1.02 **	0.61 $\pm$ 0.03 **	0.35 $\pm$ 0.02 *
TBI - II	0.48 $\pm$ 0.08 **	0.25 $\pm$ 0.02	72.3 $\pm$ 14.6 *	154.5 $\pm$ 8.3 *	9.15 $\pm$ 0.98 *	0.37 $\pm$ 0.02 *	0.26 $\pm$ 0.02 *

*
P<0.05;**
P<0.01;***
P<0.001