Temporal profile of cortical perfusion and microcirculation after controlled cortical impact injury in rats

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Abstract

Impaired cerebral perfusion contributes to evolving posttraumatic tissue damage. Spontaneous reversibility of reduced perfusion within the first days after injury could make a persisting impact on secondary tissue damage less likely and needs to be considered for possible therapeutic approaches. The present study was designed to characterize the temporal profile and impact of trauma severity on cortical perfusion and microcirculation during the first 48 h after controlled cortical impact injury (CCI). In 10 rats, pericontusional cortical perfusion and microcirculation using laser Doppler flowmetry (LDF) and orthogonal polarization spectral (OPS) imaging were assessed before, and at 4, 24, and 48 h after CCI. Influence of trauma severity was studied by varying the penetration depth of the impactor rod (0.5 vs. 1 mm), thereby inducing a less and a more severe contusion. Mean arterial blood pressure (MABP), arterial blood gases, and blood glucose were monitored. With unchanged MABP and paCO2, cortical perfusion and microcirculation were significantly impaired during the first 48 h following CCI. Hypoperfusion observed at 4 h related to vasoconstriction and microcirculatory stasis preceded a long-lasting phase of hyperperfusion at 24 and 48 h reflected by vasodilation and increased flow velocity in arterioles and venules. Hyperperfusion was mostly pronounced in rats with a less severe contusion. Following CCI, trauma severity markedly influences changes in pericontusional cortical perfusion and microcirculation. Overall, pericontusional cortical hypoperfusion observed within the early phase preceded a long lasting phase of hyperperfusion up to 48 h after CCI.
Temporal Profile of Cortical Perfusion and Microcirculation after Controlled Cortical Impact Injury in Rats

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ABSTRACT

Impaired cerebral perfusion contributes to evolving posttraumatic tissue damage. Spontaneous reversibility of reduced perfusion within the first days after injury could make a persisting impact on secondary tissue damage less likely and needs to be considered for possible therapeutic approaches. The present study was designed to characterize the temporal profile and impact of trauma severity on cortical perfusion and microcirculation during the first 48 h after controlled cortical impact injury (CCI). In 10 rats, pericontusional cortical perfusion and microcirculation using laser Doppler flowmetry (LDF) and orthogonal polarization spectral (OPS) imaging were assessed before, and at 4, 24, and 48 h after CCI. Influence of trauma severity was studied by varying the penetration depth of the impactor rod (0.5 vs. 1 mm), thereby inducing a less and a more severe contusion. Mean arterial blood pressure (MABP), arterial blood gases, and blood glucose were monitored. With unchanged MABP and paCO2, cortical perfusion and microcirculation were significantly impaired during the first 48 h following CCI. Hypoperfusion observed at 4 h related to vasoconstriction and microcirculatory stasis preceded a long-lasting phase of hyperperfusion at 24 and 48 h reflected by vasodilation and increased flow velocity in arterioles and venules. Hyperperfusion was mostly pronounced in rats with a less severe contusion. Following CCI, trauma severity markedly influences changes in pericontusional cortical perfusion and microcirculation. Overall, pericontusional cortical hyperperfusion observed within the early phase preceded a long lasting phase of hyperperfusion up to 48 h after CCI.

Key words: cerebral blood flow; contusion; intravital microscopy; laser doppler flowmetry; orthogonal polarization spectral imaging; secondary injury; traumatic brain injury

INTRODUCTION

Following traumatic brain injury (TBI), primary tissue damage is complicated by secondary injury related to a cascade of different pathophysiologically important changes resulting in structural and functional deterioration. After focal TBI, contusion size is increased during the first 48 h under clinical (Stover et al., 1999) and experimental conditions (Stover et al., 2000a). This secondary growth in contusion volume is caused by ionic...
and metabolic imbalance (Yang et al., 1985; Yoshino et al., 1991), glutamate-mediated cell damage (Katayama et al., 1990; Palmer et al., 1993), edema formation (Baethmann et al., 1988; Kempski and Volk, 1994), cell death (Sutton et al., 1993), and reduced cerebral perfusion (Bryan et al., 1995; Forbes et al., 1997; Kochanek et al., 1995; Kroppenstedt et al., 2000).

Reduction in regional cerebral blood flow (rCBF) within the traumatized cortex following CCI develops within the first 30 min after induction of contusion and remains partly impaired at 24 h after CCI as determined by autoradiographic studies (Bryan et al., 1995; Kochanek et al., 1995), perfusion magnetic resonance imaging (MRI; Forbes et al., 1997), and laser Doppler flowmetry (LDF; Cherian et al., 1994). Reduction in cortical perfusion is attributed to a multitude of different changes developing in parallel and sequentially showing a mutual dependency (Golding et al., 1999). Severe microcirculatory impairment is known to contribute to secondary tissue damage and is caused by an imbalance between vasodilating (e.g., nitric oxide) and vasoconstricting (e.g., endothelins) mediators, endothelial damage resulting in platelet and leukocyte aggregation, impaired autoregulation, and edema formation leading to compression of capillaries.

Detailed characterization of perfusion impairment via autoradiography, perfusion MRI, and stationary LDF is limited since resolution of these techniques is too low to determine changes within the microcirculation.

To characterize impaired perfusion and underlying microvascular changes more closely rCBF and cortical microcirculation were investigated by LDF using a modified scanning technique and the novel orthogonal polarization spectral (OPS) imaging, respectively.

LDF allows instantaneous, noninvasive, continuous, and repetitive analysis of changes in rCBF under in vivo conditions by calculating the Doppler frequency shift between infrared light delivered to and scattered from a tissue volume of approximately 1 mm$^3$. The Doppler frequency shift is proportional to the flow velocity of moving blood cells in the investigated tissue sample and is expressed in arbitrary laser Doppler units (LDU) (Dimagl et al., 1989; Fabricius et al., 1997). Using a stationary laser Doppler probe allows the assessment of changes in CBF restricted to a small cortical area only. With the scanning technique, however, in which a laser Doppler probe is moved in defined steps changes in cortical perfusion can be determined with a substantially increased spatial resolution as a larger region of interest is investigated (Heimann et al., 1994).

Overall, LDF, however, does not allow the differentiation between arterioles and venules. The limitation of LDF in defining whether changes in perfusion are caused by alterations in arterioles or venules can be overcome by employing the technique of intravital microscopy. The recently developed technique of orthogonal polarization spectral (OPS) imaging (Groner et al., 1999) allows the quantitation of microvascular diameter and red blood cell velocity (RBCV) of the cortical microvasculature with an intact dura and without having to administer fluorescent dyes (Thomale et al., 2001).

The aim of the present study was to investigate the temporal profile of pericontusional cortical perfusion during the first 48 h after CCI at two different degrees of trauma severity using a modified LDF scanning technique. In addition, changes in microcirculation were further characterized by OPS imaging.

**MATERIALS AND METHODS**

**Anesthesia and Trauma**

All experiments were approved by the ethics committee of the Humboldt University. Under isoflurane/N$_2$O anesthesia (isoflurane, 1.6 vol%; N$_2$O, 0.5 L/min; O$_2$, 0.3 L/min), all spontaneously breathing Sprague-Dawley rats ($n = 10$, 300–350 g) were positioned in a stereotaxic holder, and a left parietotemporal craniectomy (11 $\times$ 8 mm) was performed along the anatomical guidelines outlined by the sagittal, lambdoid, and coronal sutures and the zygomatic arch as described previously (Stover et al., 2000a). Cortical contusion was induced with the controlled cortical impact injury device using a 5-mm bolt, which was pneumatically driven with a velocity of 7 m/sec (5.2 bar). The bolt was positioned at a 45-degree angle perpendicular to the surface of the cerebral convexity at approximately 3 mm lateral to the sagittal suture. To study the impact of severity of tissue damage on pericontusional cortical perfusion, penetration depth was changed, while size of the bolt, velocity, and contact time (300 msec) remained unchanged. For a less severe trauma ($n = 5$) a penetration depth of 0.5 mm was chosen, while a penetration depth of 1.0 mm was used to induce a more severe cortical contusion ($n = 5$). In both groups, subdural hematoma developed immediately following induction of trauma, which was larger following the more severe trauma. Subdural hematoma did not allow to investigate cortical perfusion and microcirculation at the site of impact. While pericontusional cortical perfusion measurements using LDF were not influenced by the extent of subdural hematoma, pericontusional cortical microcirculation using OPS imaging could only be reliably investigated in rats with a less severe cortical contusion. The amount of subdural hematoma present in remnants by 48 h after CCI was not determined.
Physiological Parameters

The left femoral artery was cannulated to monitor mean arterial blood pressure (MABP) and to withdraw blood to determine arterial blood gases (ABG) and glucose. Repetitive cannulation of the same femoral artery before trauma, and at 4, 24, and 48 h after CCI was feasible and well tolerated by all rats. Cannulation was performed using a surgical microscope and special care was taken not to damage the femoral nerve. Rectal temperature was maintained at 37 ± 0.5°C using a homeothermic heating pad. To minimize the risk of infection, all surgical instruments were cleaned and autoclaved after each use and novel catheters were used for each cannulation.

Laser Doppler Flowmetry (LDF)

Pericontusional cortical rCBF was measured by LDF (DRT4; Moor Instruments, Devon, U.K.) and displayed in arbitrary laser Doppler units (LDU). For this, a laser Doppler needle probe with a diameter of 800 μm was attached to the micromanipulator of a stereotaxic frame at a distance of 1 mm from the intact dura. The laser Doppler needle probe was moved in 0.2-mm steps from the occipital to frontal pole over a total distance of 10 mm parallel to the sagittal suture and medial to the contusion. This procedure allowed to investigate identical areas in all rats at different time points. For each time point, 50 measurements were performed twice. Duration of one scan was approximately 5 min. Acquired data of cortical perfusion was used to assess absolute changes over time. Changes were determined based on the mean of the summed median values of each scan per rat and displayed in percent to pretrauma levels.

After assessing changes in cortical perfusion, the laser Doppler needle probe was removed and the camera used for the OPS imaging was attached to the stereotaxic frame as described previously (Thomale et al., 2001).

Orthogonal Polarization Spectral (OPS) Imaging

Cortical microcirculation was investigated by OPS imaging (Cytoscan®; Cytometrics Inc., Philadelphia, PA). With this novel technique, cortical tissue is illuminated with linearly polarized light focused on a region of approximately 1 mm in diameter and a penetration depth of 1 mm (Groner et al., 1999). Light remitted from the cortex forms an image of the illuminated region within a target of the video camera. A polarization analyzer allows only depolarized photons scattered within the tissue to pass the analyzer and contribute to the generation of the image. The wavelength is chosen within the hemoglobin absorption spectrum. Generation of the OPS image by reflected light requires scattered light for illumination and absorbed light for contrast. Thus, hemoglobin-containing structures, for example, cortical microvasculature, are visualized as dark areas, and the surrounding tissue void of hemoglobin appears in a light contrast.

The camera (length 14.5 cm; diameter of camera tip 8 mm) was attached to a micromanipulator of a stereotaxic frame allowing to move the camera within the boundaries of the craniectomy and to investigate cortical microcirculation in different regions of the pericontusional cortex (Thomale et al., 2001). Using the coordinates of the stereotaxic frame allows us to identify the same regions of interest in longitudinal studies. In each investigated rat, identical coordinates were used to study microcirculation of the parietal and frontal cortex over time.

The tip of the camera was placed within the pericontusional area at an angle of 16 degrees. The intact dura was continuously superfused with physiological saline solution preventing drying of dura. The tip of the camera covered with a special protecting plastic sheath (CytoLens®, Cytometrics Inc.) was dipped into saline solution at a distance of 2 mm over the cortical surface, providing a high-contrast resolution image of the cortical microvasculature. This working distance is essential to avoid compression of cortical vessels.

Video signals from the OPS camera were passed through a video timer and recorded on video cassettes. Off-line microhemodynamic analysis included quantification of changes in diameters and flow velocity in arterioles and venules using a computer-assisted image analysis system (Cap Image®, Dr. Zeintl, Heidelberg, Germany; Klysčz et al., 1997). Microvascular diameter was determined by measuring the inner diameter perpendicular to the vessel wall. Red blood cell velocity (RBCV) was assessed using the linear shift diagram (LSD) technique (Klysčz et al., 1997).

In addition, microvascular diameters from all rats were used to calculate normalized observational frequency histograms. Assessed values were assigned to diameter classes ranging from 0 to 80 μm in arterioles and from 0 to 105 μm in venules, respectively, before they were mathematically normalized to 100% and plotted as frequency histograms. Subdivision of diameter classes for arterioles (<20 μm, 20–40 μm, >40 μm) and venules (8–30 μm, 30–50 μm, >50 μm) were chosen according to Zweifach (1974) and Baez (1974), respectively.

Study Protocol

Changes in pericontusional cortical perfusion were assessed before and at 4, 24, and 48 h following CCI in rats with a more severe and a less severe contusion (1-
mm vs. 0.5-mm penetration depth). Presence of subdural hematoma which was more pronounced in rats with a more severe trauma did not allow to visualize cortical microcirculation through the intact dura. Therefore, temporal profile of changes in cortical microcirculation could only be investigated in rats with the less severe CCI. For each investigated time point rats were anesthetized with isoflurane anesthesia, maintaining administered isoflurane concentration at 1.6 vol% at all time points. Following each measurement, arterial catheters were removed, the scalp was sutured, and rats were returned to their cages with free access of food and water. Based on normal drinking and eating habits and movement abilities, no additional analgesics had to be applied between the measurement intervals.

**Determination of Cortical Contusion**

At 48 h following CCI, rats were killed by exsanguination and brains were removed to determine contusion volume in hematoxylin and eosin (H&E)–stained brain slices. For this, the entire brain was cut in 2-mm slices beginning at the occipital pole using a commercially available matrix for rat brain (Brain Blocker, AgnTho’s AB, Lidingö, Sweden). Following standard H&E staining, a representative section (10 μm) of each brain slice was video-recorded, and the contusion area was determined offline using a computerized image analysis system (Cap Image®, Dr. Zeintl, Heidelberg, Germany). Multiplying these average areas by the total length of measurable contusion, that is, 6 mm (more severe trauma) and 4 mm (less severe trauma) allowed us to determine the cortical contusion volume.

**Statistical Analysis**

Data are expressed as mean ± SEM. Changes over time were evaluated for statistical significance by using a one-way repeated measures analysis of variances (ANOVA). In laser Doppler flowmetry, data between the two groups were compared by using a one-way ANOVA for multiple comparisons. Statistical analysis of contusion volume between two groups was performed by Student’s t test. (Sigma Stat® 3.0; Jandel Scientific, Erkrath, Germany). LDU, vessel diameter, and RBCV are presented as mean ± SEM of the median values obtained in each rat. Differences were rated significant at \( p < 0.05 \).

**RESULTS**

**Physiological Parameters**

MABP, arterial blood gases, and blood glucose remained within physiological limits at all time points without any difference between rats with a less and more severe contusion (Table 1). Weight loss during the first 48 h after CCI was similar in all rats (less severe CCI, 2.19 ± 6.1%; more severe CCI, 2.16 ± 6.2%).

**Pericontusional Cortical Perfusion (LDF)**

Following CCI, pericontusional cortical perfusion determined by LDF was significantly decreased by 4 h after CCI compared to pretrauma levels, which was mostly sustained in rats with the more severe contusion (−62% vs. −43%, \( p < 0.05 \); Fig. 1). Over time, early hypoperfusion was followed by a long lasting period of hyperperfusion at 24 and 48 h following trauma, which was

**Table 1. Changes in MABP, Hemoglobin, Arterial Blood Gases, and Blood Glucose before and up to 48 h after CCI in Rats Subjected to a Less (n = 5) and a More Severe Injury Level (n = 5; 0.5 vs. 1.0 mm Penetration Depth; Means ± SEM)**

<table>
<thead>
<tr>
<th>Penetration depth</th>
<th>Before CCI</th>
<th>30 min</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP [mm Hg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>90 ± 1</td>
<td>93 ± 2</td>
<td>93 ± 3</td>
<td>94 ± 1</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>1 mm</td>
<td>96 ± 1</td>
<td>90 ± 2</td>
<td>89 ± 1</td>
<td>94 ± 2</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>Hb [mg/dL]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>14.2 ± 0.4</td>
<td>14 ± 0.5</td>
<td>14.7 ± 0.6</td>
<td>13.9 ± 0.5</td>
<td>14.1 ± 0.4</td>
</tr>
<tr>
<td>1 mm</td>
<td>14.3 ± 0.3</td>
<td>13.9 ± 0.4</td>
<td>13.5 ± 0.3</td>
<td>13.7 ± 1.3</td>
<td>13.5 ± 0.7</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>7.42 ± 0.02</td>
<td>7.39 ± 0.001</td>
<td>7.41 ± 0.001</td>
<td>7.44 ± 0.02</td>
<td>7.40 ± 0.02</td>
</tr>
<tr>
<td>1 mm</td>
<td>7.47 ± 0.05</td>
<td>7.44 ± 0.02</td>
<td>7.45 ± 0.02</td>
<td>7.42 ± 0.03</td>
<td>7.43 ± 0.03</td>
</tr>
<tr>
<td>paCO₂ [mm Hg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>40.3 ± 1.2</td>
<td>42.6 ± 1.2</td>
<td>41.0 ± 2.1</td>
<td>41.4 ± 3.7</td>
<td>43.6 ± 2.4</td>
</tr>
<tr>
<td>1 mm</td>
<td>36.0 ± 1.8</td>
<td>39.7 ± 1.1</td>
<td>35.6 ± 0.9</td>
<td>36.4 ± 0.9</td>
<td>41.8 ± 1.7</td>
</tr>
<tr>
<td>paO₂ [mm Hg]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>201 ± 12</td>
<td>190 ± 11</td>
<td>195 ± 20</td>
<td>220 ± 8</td>
<td>191 ± 4</td>
</tr>
<tr>
<td>1 mm</td>
<td>191 ± 16</td>
<td>184 ± 9</td>
<td>154 ± 12</td>
<td>165 ± 15</td>
<td>181 ± 12</td>
</tr>
<tr>
<td>Glucose [mg/dL]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 mm</td>
<td>124 ± 11</td>
<td>110 ± 5</td>
<td>108 ± 4</td>
<td>121 ± 10</td>
<td>142 ± 10</td>
</tr>
<tr>
<td>1 mm</td>
<td>148 ± 11</td>
<td>120 ± 9</td>
<td>131 ± 5</td>
<td>105 ± 10</td>
<td>116 ± 2</td>
</tr>
</tbody>
</table>
mostly sustained in rats with a less severe trauma (±65 and ±125%, \( p < 0.05 \); Fig. 1). Following a more severe contusion, cortical perfusion at 24 h did not differ significantly from pretrauma values (−10%), while hyperperfusion was observed at 48 h (±50%, \( p < 0.05 \); Fig. 1).

**Vessel Diameter in Pericontusional Cortical Microcirculation (OPS)**

Vessel diameter in arterioles were similar at 4 h after CCI (17.7 ± 3.2 \( \mu \text{m} \)) compared to pretrauma values (19.1 ± 2.7 \( \mu \text{m} \)). By 24 and 48 h, vessel diameter in arterioles were significantly increased by 31% and 53% (25 ± 3.5 and 29.3 ± 3.6 \( \mu \text{m}, p < 0.05 \)), respectively (Table 2).

Venular diameter remained unchanged at 4 h after CCI (24.2 ± 2.4 \( \mu \text{m} \)) compared to pretrauma levels (22.2 ± 1.4 \( \mu \text{m} \)) followed by a significant increase by 48% and 77% at 24 h (32.8 ± 4.6 \( \mu \text{m}, p < 0.05 \)) and 48 h (39.4 ± 5.1 \( \mu \text{m}, p < 0.05 \)) after CCI, respectively (Table 2).

These changes in distribution of vessel diameter of arterioles and venules were reflected by a marked shift to lower and higher values, as depicted in the normalized observational frequency histograms (Figs. 2A,B). A leftward shift to reduced diameters seen in arterioles at 4 h was followed by a rightward shift at 24 and 48 h (Fig. 2A). In venules, vessel diameter showed a rightward shift at 24 and 48 h after CCI (Fig. 2B).

**RBCV in Pericontusional Cortical Microcirculation (OPS)**

Mean RBCV in all measurable arterioles was reversibly decreased from values exceeding 5 mm/sec to 4.0 ± 0.2 mm/sec at 4 h following CCI. By 24 and 48 h after CCI, RBCV was markedly increased exceeding the measurable upper limit of 5 mm/sec. The frequency of measurable values below 5 mm/sec increased from 6% determined before CCI to 36% at 4 h after CCI, respectively. At 24 and 48 h, RBCV exceeded the measurable upper limit of 5 mm/sec in all arterioles.

In venules changes in RBCV paralleled alterations seen in arterioles. At 4 h after CCI (0.45 ± 0.06 mm/sec,

### Table 2. Changes in Vessel Diameter [\( \mu \text{m} \)] in Venules and Arterioles in Rats with a Less Severe CCI (\( n = 5 \))

<table>
<thead>
<tr>
<th></th>
<th>Before CCI</th>
<th>4 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterioles</td>
<td>19.1 ± 2.7</td>
<td>17.7 ± 3.2</td>
<td>25 ± 3.5</td>
<td>29.3 ± 3.6*</td>
</tr>
<tr>
<td>Venules</td>
<td>22.2 ± 1.4</td>
<td>24.2 ± 2.4</td>
<td>32.8 ± 4.6*</td>
<td>39.4 ± 5.1*</td>
</tr>
</tbody>
</table>

\( *p < 0.05 \) vs. pretrauma values.
$p < 0.05$) RBCV in venules was significantly reduced by 34% compared to pretrauma level (0.68 ± 0.08 mm/sec). As already seen in arterioles, RBCV in venules partially exceeded 5 mm/sec at later time points. Mean RBCV in all venules reached 3.97 ± 0.13 and 4.88 ± 0.07 mm/sec at 24 and 48 h, respectively. Overall, the frequency of measurable values below 5 mm/sec decreased from 38% to 6% at 24 and 48 h.

Representative Images of Cortical Microcirculation (OPS)

Typical findings showing pathological changes in cortical microcirculation following CCI determined by OPS imaging are shown in Figure 3. Constriction of pericontusional cortical arterioles surrounded by subarachnoid hemorrhage (Fig. 3A) and developing microcirculatory

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FIG. 2. Frequency histograms showing normalized observation frequencies of diameter classes at different time points following CCI. Class subdivision was chosen according to Zweifach for arterioles (A; Zweifach, 1974) and Baez for venules (B; Baez, 1974). A leftward shift to reduced diameters in arterioles observed at 4 h was followed by a rightward shift at 24 and 48 h. A rightward shift to enlarged diameters in venules was mostly pronounced at 24 and 48 h following CCI.
FIG. 3. Representative examples of impaired microcirculation following CCI. (A) Pericontusional cortical vessels surrounded by subarachnoid hemorrhage with constricted arterioles (4 h after trauma). (B) Microcirculatory stasis with solitary intravascular erythrocytes reflecting markedly reduced red blood cell velocity (arrows, 4 h following CCI); Spontaneous reversibility of reduction in diameter shown in the same microvessels before (C) and 4 h at (D) and 48 h (E) following trauma. Vasoconstriction in arterioles at 4 h is followed by massive dilation of arterioles and venules at 48 h after CCI.
stasis (Fig. 3B) contribute to attenuated perfusion at 4 h after CCI. Reversibility of reduced microvascular diameter in the same microvessels is depicted in Figure 3C–E, where the same arterioles and venules are shown before and at different time points following CCI. Massive dilatation of arterioles and venules reflect sustained cortical perfusion at 48 h (Fig. 3E).

**Cortical Contusion Volume**

Contusion volume was significantly increased following a more severe injury compared to rats with a less severe trauma ($47 \pm 7$ vs. $29 \pm 2$ mm$^3$; $p < 0.05$).

**DISCUSSION**

Following CCI, reversibly reduced cortical hypoperfusion preceded a long-lasting phase of hyperperfusion as determined by laser Doppler flowmetry and OPS imaging. These changes in cortical perfusion and microcirculation are most likely caused by local pathological alterations since systemic variables as MABP, paCO$_2$, pH, and hematocrit remained within physiological limits. The observed hyperperfusion does not seem related to infection since rectal temperature, MABP, arterial blood gases, blood glucose, and posttraumatic weight loss remained within physiological and acceptable limits, respectively.

As determined in a large series of experiments conducted in our laboratory designed to therapeutically influence pericontusional perfusion and cerebral perfusion pressure (CPP), intracranial pressure (ICP) increased parallel to the evolving contusion growth, reaching maximal values by 24 and 48 h, ranging from 14 to 20 mm Hg and decreasing thereafter. Resulting CPP remained above 75 mm Hg, even at 4 h after CCI when pericontusional perfusion was significantly decreased (Kroppenstedt et al., 2000), reaching lowest levels as seen in the present study.

In the present study ICP had not been measured to minimize the number of animals since positioning of an intraparenchymal Codman ICP-probe (Kroppenstedt et al., 2000; Stover et al., 2000c) is associated with dura damage and tissue injury which would require additional groups of animals for each investigated time point.

It remains to be clarified if the observed hyperperfusion contributes to increased ICP following CCI or reflects resolving elevations in ICP.

**LDF and OPS Imaging**

Scanning LDF reliably reflects changes in cortical perfusion with high spatial resolution (Heimann et al., 1994). However, this technique does not allow to characterize microcirculatory changes in arterioles and venules since the resolution of the employed needle laser Doppler probe is too low. Needle probes as used in the present study with a diameter of 800 µm generate a summed value of perfusion values determined in all microvessels present within the underlying tissue. Therefore, intravital microscopy is an indispensable tool to directly assess microcirculatory changes and differentiate between arterioles and venules (Harris et al., 2000). However, one setback of investigating cortical microcirculation is the inability to determine high RBCV values in arterioles and venules. Using the linear shift diagram technique (Klyscz et al., 1997) flow velocity in cortical microvessels cannot be measured once it exceeds an upper detection limit of 5 mm/sec as in the present study. Thus, exact RBCV values could only be determined at 4 h after CCI in venules. However, calculating the frequency of measurable values allows to assess changes in RBCV at different time points after trauma in arterioles and venules.

Due to technical reasons changes in pericontusional cortical microcirculation could not be determined in the same areas as investigated by laser Doppler flowmetry. Since the tip of the OPS camera is 8 mm in diameter, it must be positioned at the lateral hemispheric convexity to avoid interference with the boundaries of the craniectomy. Best results are obtained by LDF if the laser Doppler needle probe is positioned at a 90-degree angle to the surface medial to the contusion site. Therefore, changes in perfusion and microcirculation were determined in different pericontusional cortical structures, that is, medial (LDF) and lateral (OPS) to the contusion.

**Posttraumatic Hypoperfusion and Hyperperfusion**

Reduced cerebral perfusion contributing to evolving secondary brain injury is a common finding following clinical (Martin et al., 1997) and experimental (Bryan et al., 1995; Cherian et al., 1996; Kroppenstedt et al., 1999, 2000) traumatic brain injury. This decrease in perfusion is caused by several different factors which are activated independently and are known for their mutual dependency. Among others, thrombotic occlusions mediated by activation of platelet activating factor (PAF; Hekmatpanah and Hekmatpanah, 1985; Dietrich et al., 1996; Maeda et al., 1997), and obstruction of vessels by edema formation, petechial hemorrhage and extraluminal clots (Hekmatpanah and Hekmatpanah, 1985) as well as an imbalance between dilating and constricting factors (Golding et al., 1999) lead to impaired cerebral perfusion.

Nitric oxide is the predominant vasodilating mediator. Immediately after traumatic brain injury, fast upregulation of constitutive nitric oxide synthase (cNOS; Wada et al., 1998) coincides with increased extracellular NO.
(Cherian et al., 2000) and NO degradation products nitrite and nitrate (Rao et al., 1998). Over time, however, NO synthesis is diminished as reflected by reduced extracellular NO (Cherian et al., 2000), nitrate, and nitrite concentrations (Rao et al., 1998) as well as decreased cNOS activity (Wada et al., 1998). Infusing l-arginine, the precursor of NO, significantly reverses impaired posttraumatic cortical perfusion (DeWitt et al., 1997, Cherian et al., 1999) which, in turn, suggests that during the early period following traumatic brain injury NO is insufficiently synthesized or excessively degraded. A decrease in cNOS located on endothelial cells and neurons appears to contribute to the observed decrease in cortical perfusion and reduced vessel diameter at 4 h after CCI. The subsequent increase in LDF, vessel diameter and microcirculatory perfusion values by 24 and 48 h suggest restored vasodilatation. In this context, expression of the inducible isoform of NOS (iNOS) has been shown to be significantly increased as of day 3 on astrocytes and macrophages within the pericontusional cortex (Wada et al., 1998).

Vasoconstriction is predominantly mediated by endothelin-1, which is significantly increased in CSF during the early phase following traumatic brain injury (Armstead, 1996). Pharmacological inhibition significantly prevented posttraumatic endothelin-1-mediated constriction of small pial arteries and arterioles (Armstead, 1996).

In addition, release of hemoglobin from lysed erythrocytes contributes to vasoasm, thus reducing rCBF and leading to delayed ischemia and cortical necrosis (Dreier et al., 2000). Furthermore, increased leukocyte sticking and plugging contribute to reduced perfusion and developing microcirculatory stasis as described in the early phase following a focal traumatic lesion in rabbits (Hartl et al., 1997).

By 24 and 48 h following CCI cortical hyperperfusion, a sign of hyperemia coincided with significant increases in vessel diameter and RBCV in arterioles and venules despite unchanged paCO2, MABP, and hematocrit. As shown under clinical (Martin et al., 1997) and experimental settings (Assaf et al., 1999; Kelly et al., 2000), reversible cerebral hyperemia is present between 24 and 72 h following traumatic brain injury and proceeds early posttraumatic hypoperfusion. To date, the exact reasons for this transient hyperemia remain unclear. Apart from vascular smooth muscle dysfunction leading to vasospasm (Gomez et al., 1991), sustained release of vasodilating mediators, for example, NO, bradykinin (Cherian et al., 2000, Iadecola et al., 1994, Unterberg et al., 1984) K+, and adenosine, as well as tissue acidosis (Golding et al., 1999) could contribute to the observed hyperperfusion. It remains to be clarified if CO2 reactivity, which is decreased as early as 30 min after CCI (Golding et al., 1999) and remains disturbed up to 24 h (Forbes et al., 1997), is restored by 48 h, thereby increasing the influence of vasodilating mediators and possibly contributing to the observed hyperperfusion. Specific modulation of these mediators at later time points following traumatic brain injury could be of therapeutic value in attenuating this hyperemic response which is thought to contribute to increased intracranial pressure and an unfavorable outcome in some brain-injured patients (Kelly et al., 1996).

Severity of traumatic brain injury is decisive for the development of evolving structural and functional deterioration and also influences efficacy of neuroprotective drugs (Stover et al., 2000a). Increasing severity of traumatic brain injury results in a graded decrease in cortical perfusion during the early phase following CCI (Cherian et al., 1994; and as observed in the present study). Interestingly, rats subjected to a less severe trauma showed a more pronounced hyperemic response as early as 24 h following CCI compared to those rats receiving a more severe injury. It remains to be clarified if the lesser extent of tissue damage and a more preserved reactivity to vasodilating mediators contribute to the observed sustained hyperperfusion.

Overall, contribution of impaired pericontusional cortical perfusion and microcirculation to evolving tissue damage seems limited to the early phase following CCI since reduced perfusion is reversible by 24 h after CCI, a time point at which secondary growth of contusion and edema formation reaches its maximal extent (Stover et al., 2000b). The observed hyperperfusion does not seem to contribute to evolving tissue damage since it was mostly pronounced in rats with a significantly smaller contusion.

CONCLUSION

The present study characterized the time course of changes in cortical perfusion during the first 48 h following CCI. During the early phase, cortical hyperperfusion preceded a long-lasting phase of hyperperfusion. These changes, determined by LDF, were verified by OPS imaging. Identification of reasons accounting for these characteristic alterations in rCBF and possible implications for temporally adapted therapeutic strategies warrant further investigation.

REFERENCES


CORTICAL PERFUSION AFTER CCI


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