Platelet-activating factor and progressive brain damage following focal brain injury

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The effects of a platelet-activating factor (PAF) antagonist on brain edema, cortical microcirculation, blood-brain barrier (BBB) disruption, and neuronal death following focal brain injury are reported. A neodymium:yttrium-aluminum-garnet (Nd:YAG) laser was used to induce highly reproducible focal cortical lesions in anesthetized rats. Secondary brain damage in this model was characterized by progressive cortical hypoperfusion, edema, and BBB disruption in the vicinity of the hemispheroid lesion occurring acutely after injury. The histopathological evolution was followed for up to 4 days. Neuronal damage in the cortex and the hippocampus (CA-1) was assessed quantitatively, revealing secondary and progressive loss of neuronal tissue within the first 24 hours following injury.

Pretreatment with the PAF antagonist BN 50739 ameliorated the severe hypoperfusion in 12 rats (increasing local cerebral blood flow from a mean ± standard error of the mean of 40.5% ± 8.3% to 80.2% ± 7.8%, p < 0.01) and reduced edema by 70% in 10 rats (p < 0.05) acutely after injury. The PAF antagonist also reduced the progression of neuronal damage in the cortex and the CA-1 hippocampal neurons (decrease of neuronal death from 88.0% ± 3.9% to 49.8% ± 4.2% at 24 hours in the cortex and from 40.2 ± 5.0% to 13.2% ± 2.1% in the hippocampus in 30 rats; p < 0.05).

This study provides evidence to support progressive brain damage following focal brain injury, associated with secondary loss of neuronal cells. In this latter process, PAF antagonists may provide significant therapeutic protection in arresting secondary brain damage following cerebral ischemia and neurological trauma.

KEY WORDS • brain injury • platelet-activating factor • laser • ischemia • cerebral blood flow • rat

Several different pathophysiological events are thought to underlie secondary tissue damage following craniocerebral trauma. These include the extracellular release of excitatory neurotransmitters and free fatty acids, the intracellular accumulation of calcium, generation of free radicals, and synthesis of eicosanoid products. These factors are believed to contribute to vasogenic and cytotoxic edema, disruption of the blood-brain barrier (BBB), impaired microcirculation, and ultimately to secondary loss of neuronal tissue. No effective therapy has been suggested to halt this secondary deterioration following traumatic brain injury.

Recently, interest has been focused on the role of platelet-activating factor (PAF) in neurological injury. Originally isolated from stimulated basophils, PAF can be produced by a variety of cells such as neutrophils, platelets, monocytes, macrophages, and endothelial cells; it has also been suggested as a mediator of inflammation, platelet and neutrophil activation, plasma extravasation, and anaphylactic shock. Platelet-activating factor is synthesized by the sequential activities of the phospholipase A2 and acetyltransferase, which depends on cell activation and the presence of calcium. PAF is inactivated by acetylhydrolase and acyltransferase, which is independent of cell stimulation. Alkyl-acyl glycerophosphocholine, which is stored in cell membranes, is not only the end-product of the cellular metabolism of PAF, it is also the potential precursor of PAF as well as of arachidonic acid.

The involvement of PAF in the pathophysiology of central nervous system (CNS) disorders has been suggested in several studies. Platelet-activating factor increases the BBB permeability and reduces cerebral blood flow (CBF) in the cortex presumably by constriction of cerebral vessels. Beneficial effects of PAF antagonists have been shown repeatedly in various models of cerebral ischemia. Thus, pretreatment as well as postsischemic administration of the PAF antagonist BN 52021 resulted in reduction of edema formation.
and improved neurological outcome following global ischemia in the gerbil. In the same model, administration of a PAF antagonist upon reperfusion resulted in improved cerebral microcirculation in the phase of hypoperfusion; furthermore, the pathological postischemic accumulation of free fatty acids was reduced by PAF inhibition. These effects were not associated with any alterations of tissue phosphoinositides or diacylglycerol, suggesting that the phospholipase A2 might be an important mediator of PAF-related effects. Additional support for the potential role of PAF in mediation of brain damage can be drawn from recent findings showing the existence of PAF in brain tissue and elevated PAF levels following electroconvulsive disorders.

Previous reports on the interaction of the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser with nervous tissue indicated the eventual usefulness of this laser for production of highly reproducible focal cortical lesions. Edema, BBB disruption, selective neuronal necrosis (which develop beyond the boundary of the edematous zone), and the evolution of the histopathological picture suggest that Nd:YAG laser-induced brain lesions may be appropriate for studying mechanisms of secondary brain damage following traumatic brain injury. The present study has been designed to further characterize the evolution of widespread brain injury in response to focal trauma and to investigate the potential role of PAF in this process.

Materials and Methods

Surgical Preparation

The experiments were carried out on 102 barrier-reared male Sprague-Dawley rats, each weighing from 280 to 340 gm. The animals were housed at a controlled temperature of 22°C, with a light/dark cycle of 12 hrs/12 hrs and free access to food and water. All animals were treated in accordance with the principles set forth in the "Guide for the Care and Use of Laboratory Animals," 1985.

Anesthesia was induced by an intraperitoneal injection of 12 mg ketamine plus 0.12 mg acepromazine/100 gm body weight. Following depilation of the scalp, the calvaria was exposed by a midline scalp incision. For qualitative and quantitative histopathological studies, the dura of the right parietal cortex was exposed in rats by a 3 x 3-mm craniotomy using a high-speed drill under the operating microscope. All experimental animals were then subjected to focal brain injury by an Nd:YAG laser beam directed at the cortex. Subsequently, the removed bone flap was repositioned and the incision approximated with 4-0 silk. The animals were separated into groups that were allowed to recover for periods ranging between 4 minutes and 4 days after injury. For evaluation of BBB integrity, polyethylene (PE 50) tubing was inserted into the left femoral vein of 10 rats. To facilitate measurement of local CBF, the size of the craniotomy was enlarged to 8 x 3 mm in 17 rats and PE 50 tubing was inserted into the left femoral artery for monitoring the mean systemic arterial blood pressure (MABP). For assessment of brain water content, bilateral symmetrical parietal craniotomies were performed (8 x 3 mm each). Edema, BBB integrity, and CBF were followed for up to 2 hours after injury. During surgery and recovery, the body temperature of all animals was monitored and maintained at 37.5°C by a heating pad.

Trauma Induction

Focal brain injury was induced with an Nd:YAG laser transmitted through a fiberoptic delivery system, which was attached to a micromanipulator. The Nd:YAG laser was aimed stereotactically 3.6 mm posterior to the bregma and 3 mm lateral to the midline, guided by the internal low-power helium-neon laser aiming beam. The exposed dura was irradiated perpendicularly from a 10-mm distance with a 1-second pulse using incident powers of 40 W for study of CBF, BBB, and tissue water content and preliminary histopathological examination, and of 20 W for quantitative histopathological study of PAF antagonist effects versus control results; thus a total energy of 40 J and 20 J was delivered, respectively. At the tissue level, the diverging Nd:YAG laser had a spot diameter of 2.5 mm based on the assessment of the energy profile of the laser beam. The basic surgical procedure and trauma induction lasted approximately 10 minutes.

Treatment Protocol

The PAF antagonist BN 50739 was solubilized in dimethyl sulfoxide (DMSO), 64%, to reach a concentration of 10 mg/ml. In all experimental animals, BN 50739 was administered intraperitoneally in a dose of 10 mg/kg body weight as pretreatment 30 minutes before injury. Pharmacodynamic studies from this laboratory suggest that the protocol described provides sufficient blocking capacity of PAF-induced hypotension for over 5 hours in rats. Control animals received only the DMSO vehicle. The antagonist BN 50739 belongs to the family of PAF antagonists formed by ginkgolides, which inhibit the activities of PAF on the receptor level.

Local Cerebral Blood Flow Measurement

Local CBF was assessed continuously on-line using laser-Doppler flowmetry (LDF). The validity and usefulness of this method for measuring blood flow in the CNS both for experimental and clinical.
applications has been shown in several studies. Under physiological and pathophysiological conditions, laser-Doppler blood flows in the rat cortex correlated well with simultaneous measurements of cortical CBF using the hydrogen clearance method.\textsuperscript{34} Local CBF was measured with a flexible endoscopic flow probe,\textsuperscript{§} which was attached to a micromanipulator in order to allow accurate positioning of the probe and diminish movement artifacts. The probe was connected to a separate blood perfusion monitor\textsuperscript{¶} continuously emitting a 2-mW laser diode beam (780 nm wavelength). The backscattered light, which was Doppler-shifted by moving red blood cells, was detected by photo tubes in the same probe for real-time computing of the frequency shift to establish the flow estimate on-line: this finding was directed as a voltage signal to a pen-chart recorder. Experimental studies as well as theoretical considerations suggest that this technique permits assessment of the cerebral microcirculation in a tissue block of about 1 cu mm.\textsuperscript{11}

In 17 rats before injury, an operating microscope was used to position the flow probe perpendicular to the dura and directly adjacent to the laser aim beam (Fig. 1); local CBF was then monitored. Since the diameter of the laser aim beam was 2 mm and the diameter of the flow probe was 2 mm, local CBF was measured within a 2-mm distance from the lesion center. This group of animals was subdivided into three groups: 1) seven vehicle-treated control rats; 2) five rats with PAF antagonist pretreatment; and 3) five sham-operated control rats (craniotomy only). In all subgroups, CBF was monitored prior to and up to 2 hours after injury.

**Tissue Water Content Assessment**

In 10 animals (five each in a treatment and control group), the brain-tissue water content was measured in the vicinity of the lesion (Fig. 1) and in the corresponding area of the contralateral hemisphere, as described previously.\textsuperscript{32} Two hours after injury, 3 × 2 × 3-mm tissue cubes weighing 22.2 ± 2.4 mg were excised from the parietal cortex directly adjacent to the lesion core. There was no significant difference in the tissue sample weights of the control group compared with the treatment group. The wet weight (WW) was rapidly measured with a chemical balance.\textsuperscript{*} The tissue was then dried in a desiccator oven\textsuperscript{†} at 110°C for 48 hours to reach constant dry weight (DW). The tissue water content was calculated as (WW - DW)/WW.

**Blood-Brain Barrier Permeability**

Blood-brain barrier integrity was assessed in 12 rats (seven in a treatment group and five in a control group) by intravenous injection of Evans blue dye (2% in saline, 0.1 ml/100 gm body weight) 30 minutes before rapid removal of the brain at 2 hours after injury. Photomicrographs of the lesion area were obtained immediately after brain removal with the aid of a stereoscopic zoom microscope at × 2 magnification in order to measure the inner (ID) and outer diameter (OD) of the Evans blue dye extravasation ring on the surface of the parietal cortex. The area (in square millimeters) was calculated as \( \pi \times (\text{OD}/2)^2 - \pi \times (\text{ID}/2)^2 \).

**Qualitative and Quantitative Histopathology**

Twenty-five rats in the 40-W radiation group were sacrificed (five each time) at 4 minutes or 2, 8, 24, or 96 hours after injury, then studied histopathologically. In studies utilizing the PAF antagonist, 30 rats (15 in a control and 15 in a treatment group) received the 20-W radiation protocol. Groups were examined histopathologically after sacrifice at 4 minutes, 8 hours, or 24 hours after trauma.

The procedure was as follows. At the designated time point, a rapid thoracotomy was performed (under ketamine/acepromazine anesthesia) and the animals were perfused through the left cardiac ventricle with 150 ml normal saline, containing 10 IU heparin/ml, followed by 150 ml 10% buffered formalin (pH 7.2). The brains were kept in 20 ml fresh fixative overnight. On the following day, the brains were embedded in paraffin and sliced coronally throughout the lesion area. At each 60-μm level, one 10-μm and two 6-μm sections were taken. The 10-μm section was stained with Luxol fast blue and counterstained with cresyl violet, one 6-μm section was stained with hematoxylin and eosin (H & E), and one 6-μm section remained unstained. The level of maximum extension of the hemispheroid lesion was then identified microscopically by measuring the diameter of the well-demarcated boundaries for further qualitative and quantitative evaluation.

The neuronal damage was assessed quantitatively in representative areas near the lesion (parietal cortex) and in the CA-1 sector of the hippocampal formation of...
the ipsilateral hemisphere. This measurement was carried out in a standardized fashion as follows: Photomicrographs (magnification \( \times 10 \)) of the histological sections were taken at the level of maximum lesion extension. On enlarged color prints, the boundaries of the lesion (A and B, Fig. 2) were assessed in order to determine the geometrical midpoint (M) of the lesion. The position of the coordinates A and B underwent changes as the histological picture progressed. This, however, did not affect the position of M, which served as the reference point to define the median areas of neuronal counting in the hippocampus and cortex, if it was assumed that the histopathological changes occurred symmetrically on each side of the lesion’s midline (m, Fig. 2). To determine a standardized area (y) in the CA-1 sector, line m was drawn through point M perpendicular to AB. At the cross point of m and the CA-1 region, neuronal damage was assessed microscopically (magnification \( \times 160 \)) in a 0.625-mm length, using an eyepiece micrometer disc to delimit y.

To determine a standardized field x (Fig. 2) in the parietal cortex beyond and lateral to boundary B of the lesion, a line was drawn perpendicular to AB at a fixed distance (40-W protocol group: line MD = 1.7 mm and 1.62 mm, to yield two microscopic fields with different degrees of neuronal damage; 20-W protocol group: line MD = 1.42 mm) from reference point M. This line defines the center of the field x, directly adjacent to the cortical surface. Neuronal damage was assessed in an area of 0.307 sq mm (magnification \( \times 160 \)) using the eyepiece crosshair micrometer disc to identify the center and to delimit the borders of the field x, thereby encompassing cortical neurons of layers I to IV.

Analogous fields in the contralateral hemisphere were determined to obtain intra-individual neuronal control counts. This was done by copying the reference point M to the contralateral side (M,) (Fig. 2). The interhemispheric line c was used as the mirror plane. The plane angle (\( \alpha \)) at the intersection of c and the line AB was translated to the contralateral side and the position of M, was determined in the same distance from C as M from C. Areas of neuronal counting were then defined symmetrically by the same procedure as for the ipsilateral hemisphere.

Neuronal damage was assessed by histopathological criteria and grading scales as in former studies.\(^{15,25,26}\) In the present investigation, we classified the state of the neurons according to a two-grade system: Grade 1 neurons were normal or moderately damaged and did not show light microscopic signs of irreversible cell death as described for Grade 2; and Grade 2 neurons were irreversibly damaged, exhibiting severe shrinkage, pyknotic nuclei, cytoplasmic eosinophilia, and/or incrustations as the light microscopic correlates of cell death. These signs of irreversible neuronal damage could be most readily distinguished from stages of reversible cell damage and neurons that appeared normal. Neuronal damage was expressed as the percent cell death per representative field, calculated as:

\[
\text{Grade 2 neurons} \times 100.
\]

The intraparenchymal accumulation of neutrophils was quantified by counting in the same fields in which neuronal damage was evaluated. Neutrophils were identified by their characteristic shape. Segments as well as band neutrophils could be readily distinguished in the H & E- and Luxol fast blue-stained sections.

The geometrical determination of the representative fields of neuronal counting and the light microscopic grading and counting of neurons in the specified fields were carried out by an investigator who was blinded to both the treatment protocol and survival time.

**Statistical Analysis**

All data are expressed as the mean \( \pm \) standard error of the mean number of rats indicated. Local CBF, neuronal damage, and neutrophil accumulation were tested by Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. Tissue water content, Evans blue dye extravasation, body weight, and the stereotactical position of the lesion center were tested by Student's \( t \) statistics. Differences in neuronal damage at various distances from the lesion center were tested by Wilcoxon matched pairs test. \( P \) values less than 0.05 were accepted as statistically significant.

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**Fig. 2.** Diagram showing factors in the determination of the standardized areas for evaluation of neuronal damage. From the section with the maximum lesion diameter, areas x and y were determined geometrically on enlarged color prints of the coronal brain section as shown and described in the text. The reference midpoint M, independent of changes in the standardized areas for evaluation of neuronal damage.

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Results

Histopathological Evolution in Untreated Control Rats

At 4 minutes after injury, a well-demarcated hemisphere necrotic lesion core was surrounded by a paler-stained outer rim, exhibiting a rarified neuropil (Fig. 3A). Arterial and venous thrombosis could be observed in the lesion core as well as in pial vessels overlying the lesion area. At 2 hours after injury, the boundaries of the lesion had considerably expanded. Fibrin and red blood cell (RBC) extravasation could be found in the outer rim. At 8 hours after injury, neutrophils (Fig. 3C and D) had started to infiltrate the lesion, and fibrin and RBC extravasation had increased. At 24 hours after injury, neutrophil accumulation had reached its maximum and small hemorrhages could be found in the outer zone. Four days after injury, scar formation was manifested by neovascularization and gliosis in the outer zone, accompanied by the appearance of macrophages.

Local Cerebral Blood Flow

There was no significant difference in local CBF between the baseline levels of the control group (0.30 ± 0.04 V) as compared to the BN 50739-treated group (0.38 ± 0.06 V). Up to 10 minutes after injury, variable and nonsignificant changes were observed in the microcirculation in the vicinity of the lesion in both the control and the treatment group compared to the sham-operated group (Fig. 4). One hour after injury, substantial hypoperfusion gradually developed in the control group in which blood flows measured by LDF were reduced to a mean ± standard error of the mean of 54.2% ± 9.9% (p < 0.05 compared to flows in the sham-operated group) while the BN 50739-pretreated animals exhibited only a moderate reduction of local CBF to 74.8% ± 6.6%. The difference in flow between the control and pretreated animals was not significant at 1 hour whereas at 2 hours after injury CBF declined further in the control group (40.5% ± 8.3%: p < 0.01 compared with the sham-operated group and p < 0.05 compared with the BN 50739-treated group). At 2 hours, the BN 50739-treated group already showed a tendency for recovery (80.2% ± 7.8%). The microcirculatory changes in the pretreatment group were not significantly different from those in the sham-operated rats at any time posttrauma.

The MABP did not change significantly in any group during the entire observation period.

Fig. 3. Photomicrographs of brain tissue in untreated rats, at 4 minutes (A and B) and 8 hours (C and D) after injury. A: Lesion area 4 minutes after injury exhibiting a necrotic core surrounded by an edematous rim (arrows). Thrombotic vessels in the coagulated lesion core are indicated by arrowheads. H & E, ×17. B: Area in the uninjured contralateral hemisphere, which mirrors the area shown in C and D in the proximity of the lesion, showing normal neuronal and vascular tissue. H & E, ×427. C: Intravascular accumulation and extravasation of neutrophils (arrows) as seen in the vicinity of the lesion core within the edematous rim 8 hours after laser trauma. H & E, ×270. D: Neutrophil (arrow) in close spatial relationship to pyknotic neuron (arrowhead) 8 hours after injury. H & E, ×427.

Fig. 4. Cortical cerebral blood flow (CBF) response in the vicinity of the lesion (LDF = laser-Doppler flow). The CBF in the untreated traumatized control group showed progressive hypoperfusion acutely after injury. There were significant differences between the control group and the sham-operated group at 1 hour (* = p < 0.05) and 2 hours (** = p < 0.01) after injury. Platelet-activating factor inhibition markedly improved the altered microcirculation with a significant difference between the findings of the pretreated and the untreated group 2 hours after laser trauma (+ = p < 0.05).
Blood-Brain Barrier Permeability

Evans blue dye extravasated immediately upon trauma induction in both the pretreated group and the untreated control group, appearing as a ring on the cortical surface surrounding the lesion core. The superficial ring of Evans blue dye expanded macroscopically within the first 2 hours. At 2 hours postinjury, the OD and the ID of the ring of Evans blue dye in the pretreated rats (OD 4.38 ± 0.08 mm, ID 2.22 ± 0.07 mm) were found to be smaller than those in the control group (OD 4.78 ± 0.08 mm, ID 2.51 ± 0.04 mm; p < 0.05). Yet, at 2 hours, the area of Evans blue dye extravasation in the control rats was considerably larger (13.03 ± 0.7 sq mm) than in the BN 50739-pretreatment group (11.2 ± 0.5 sq mm, p < 0.05).

Tissue Water Content

Two hours after trauma, tissue water content adjacent to the lesion core was increased (82.4% ± 0.6%) compared to the homologous area of the contralateral hemisphere (80.2% ± 0.5%, p < 0.05) assessed at the same time (Fig. 5). The tissue water content in the contralateral hemisphere of untreated control animals was found to be in good agreement with control values in previous studies (80.1% ± 0.3%), where a similar tissue sampling technique was used. Tissue water content in the lesion hemisphere of the BN 50739-pretreatment group was significantly reduced (80.5% ± 0.5%, p < 0.05) compared to the control group and was not significantly different from the water content in the corresponding area of the contralateral hemisphere (79.9% ± 0.5%).

Quantitative Histology and Assessment of Neuronal Damage

The evolution of neuronal damage in a group of untreated control rats is shown in Fig. 6. Neuronal damage was assessed at two distances from the lesion center M (see Fig. 2). At the shorter distance (1.62 mm), neuronal death was observable by 4 minutes after injury, reaching almost 100% 24 hours later. The progression of neuronal damage was significant during the course of the 1st day after the lesion, but no additional damage was observed at 96 hours. The question raised by these data was whether 24 hours constituted the endpoint of maximum neuronal damage in our model, since further progression at the chosen distance was impossible to assess due to the already complete damage. Therefore, the area of neuronal counting was moved to 1.70 mm, at which distance the same pattern of evolution could be observed with consistently less damage (p < 0.05) at each time point compared individually to the closer microscopic field. Neuronal death reached its maximum, but not 100%, at 24 hours after trauma (84.4% ± 5.3%) without any further progression at 96 hours, suggesting that maximum damage was reached at 24 hours after injury.

Effect of BN 50739 on Neuronal Death and Neutrophil Accumulation

Based on the above series of experiments, three representative time points were chosen for evaluation of the effectiveness of BN 50739: 4 minutes, 8 hours, and 24 hours after injury. There was no significant difference in the stereotactic position of the lesion between the control animals (bregma -3.67 ± 0.08 mm, midline 3.02 ± 0.04 mm) and the treatment group (bregma -3.67 ± 0.05 mm, midline 3.1 ± 0.06 mm) nor in the body weight of the two groups (control group 308 ± 5 gm, treatment group 310 ± 5 gm).

![Graph](Image)

**Fig. 5.** Effect of the platelet-activating factor antagonist BN 50739 on edema formation. The tissue water content in the lesioned hemisphere of the untreated control group was substantially increased compared to the contralateral sides (+ = p < 0.05). Pretreatment with BN 50739 blocked the pathological tissue water accumulation in the lesioned hemisphere. There was a significant difference between the lesioned hemispheres of the control and the pretreated animals (* = p < 0.05).

![Graph](Image)

**Fig. 6.** Evolution of neuronal death in the cerebral cortex. Neuronal death was assessed at two distances from the lesion center. At the closer range (1.62 mm), neuronal cell loss progressed to 100% by 24 hours after injury. Neuronal damage increased significantly between 4 minutes and 8 hours, between 2 and 24 hours, and between 8 and 24 hours after injury. To evaluate whether neuronal death might further progress after 24 hours, neuronal death was assessed in a more remote area at a distance of 1.70 mm from the lesion center. The same pattern of progression of neuronal death was observed and 24 hours appeared to mark the point of ultimate extent of neuronal damage.
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Figure 7 demonstrates the evolution of neuronal damage in the cerebral cortex in these experiments. The control group displayed a familiar pattern of progressive neuronal cell loss during the 24-hour observation period, while in the pretreated animals, progression of neuronal damage could be observed between 4 minutes (7.9% ± 0.6%) and 8 hours (42.6% ± 5.3%, p < 0.01), and no further significant increase was found at 24 hours (49.8% ± 4.2%). Neuronal damage was significantly different between the control and the treatment group at 8 hours (p < 0.05) and at 24 hours (p < 0.01).

Figure 8 demonstrates the evolution of neuronal damage in the CA-1 sector of the hippocampus. No damage could be observed at 4 minutes after injury in either group. Neuronal cell death in the control group progressed between 8 and 24 hours (p < 0.05). Pretreatment with BN 50739 reduced hippocampal neuronal cell loss at 8 hours compared to approximately 50% of the control group (p < 0.05) and blocked further progression after that time.

Figure 9 demonstrates the results of neutrophil counting in each field examined for the evaluation of neuronal damage. Neutrophil accumulation progressed significantly between 8 hours (11 ± 1 hours) and 24 hours (23 ± 3 hours, p < 0.05) in the control group. There was less intraparenchymal neutrophil accumulation in the pretreated group at 8 hours (8 ± 2 hours), with no significant increase by 24 hours (13 ± 4 hours).

Discussion

Secondary Brain Damage in Nd:YAG Laser-Induced Focal Brain Injury

The Nd:YAG laser is widely used in neurosurgery and is known for its hemostatic properties. The Nd:YAG laser is mostly absorbed in blood and blood components and causes tremendous temperature swings spreading beyond the boundaries of the lesion spot center. These studies indicated that heat damage may be the most prominent mechanism in the interaction of the Nd:YAG laser with neural tissue. Therefore, the thrombotic events and histological appearance of the central lesion core were most likely caused by thermal coagulation. The surrounding area beyond the lesion core, however, featured characteristics of secondary brain injury, such as BBB disruption and edema formation, which have also been noticed in previous studies using the Nd:YAG laser for experimental nervous tissue injury.

In the present model, severe hypoperfusion in the proximity of the lesion accompanied substantial edema formation and increased BBB permeability, which are considered to be important mechanisms of secondary brain damage. These secondary events were associated with a progressive loss of neuronal tissue in a spatial interrelationship. Figure 1 illustrates schematically the spatial interrelationship of the histological boundaries of the lesion (as seen on the cortical surface) with the areas of CBF measurement, edema assessment, and neuronal counting. The area of CBF measurement...
overlapped the area of neuronal counting, which was beyond the initial boundary of the lesion. Both areas were encompassed by the tissue block, which had been excised for determination of the tissue water content, and within the extravasation ring of Evans blue dye. Therefore, our data seem to suggest that therapeutic efforts to preserve injury brain tissue could be assessed indirectly by their effects on edema formation, CBF, and BBB integrity, since hypoperfusion and concurrent edema formation and BBB disruption were interrelated with progressive secondary loss of nervous tissue.

**Edema and Blood-Brain Barrier.** In the present model and in other models of focal brain injury, edema is a consistent manifestation in traumatic neurological injury. Similar tissue water content in control rats was reported by Chan, et al., using the same technique to obtain tissue samples in cold-injured rat brain. As an indicator of the BBB integrity, Evans blue dye outlines potential pathways for plasma components, such as calcium, glutamate, fatty acids, kinins, and prostaglandins, which could eventually contribute to neuronal cell death. The pattern of dye extravasation in the cold lesion appears to be slightly different from that in the heat-injured brain. In cold-injured brain, Evans blue dye fluorescence is initially found throughout the entire lesion, but is lost in the lesion core by 24 hours after injury, evidently due to stasis in this area. Our own results, in agreement with other reports on the effects of heat damage on the BBB, showed an immediate BBB disruption without any Evans blue staining in the lesion core, which had apparently undergone coagulation necrosis.

**Microcirculatory Changes.** Delayed posttraumatic hypoperfusion has also been observed in other models of traumatic brain injury. In order to rule out the possibility that these changes were specific to the type of injury, Pappius compared the effects of cold as well as heat injury on brain glucose metabolism and blood flow. Her findings supported the suggestion that hypoperfusion develops in the vicinity of the focal lesion and is accompanied by decreased glucose utilization in the cold as well as the heat lesion. Interestingly, glucose utilization was found to be reduced even in remote brain areas unrelated to blood flow changes, which exhibited rather hyperemic changes.

**Histopathology and Progression of Neuronal Damage.** The histological evolution in the present study was in good agreement with that observed in other models of focal brain injury. The early occurrence of extravasation of erythrocytes and the more delayed onset of small hemorrhages in the present and other traumatic injury models are pathophysiologically important phenomena resembling human pathology of focal brain trauma. The occurrence of inflammatory cells was also observed in the cold-lesion model, most prominently at 48 hours after injury.

We are not aware of other reports in which neuronal tissue damage has been quantitatively assessed and correlated with the formation of edema, BBB disruption, and postinjury hypoperfusion in the vicinity of a focal brain lesion in such a highly reproducible fashion. The temporal evolution of the loss of neuronal tissue following traumatic brain injury as established in the current model may bear clinical relevance, since the delayed progressive tissue destruction could provide therapeutic time windows after the original insult.

**Platelet-Activating Factor Inhibition in Neurological Injury**

No study to date has investigated the role of PAF in experimental traumatic brain injury. Pretreatment with the potent and selective PAF antagonist BN 50739 almost completely blocked edema formation in the environs of the lesion and diminished the area of increased BBB permeability by retracting the outer border of Evans blue dye extravasation. The zone of the nonstained central core was found to be smaller, possibly indicating perfusion in an area which, without treatment, would not be perfused at all. To support this conclusion, further studies are needed to assess the total amount of extravasated Evans blue dye on the cortical surface. The deterioration of the cerebral microcirculation was markedly improved by PAF inhibition, and cortical CBF showed a continuing tendency to increase at 2 hours after injury, not being significantly different from the sham-operated group. In the present model, intraparenchymal neutrophil accumulation estimated by direct counting in histological sections was significantly reduced at 24 hours after injury in the BN 50739-treated group.

Improved microcirculation, reduced edema formation, decreased BBB permeability, and reduced neutrophil accumulation were correlated with an improved survival of neuronal tissue in the cerebral cortex and the hippocampus, thereby confirming the hypothesis of the secondary nature of the progressive tissue damage in this model. Neuronal death was most prominently reduced at the 24-hour sampling point in the BN 50739-pretreated animals. Microcirculatory improvement, reduction of edema formation, and changes in the BBB permeability were observed at early time points, but might have been of great importance for the improved neuronal survival later on. Further studies on the effectiveness of the curative application of PAF antagonists subsequent to traumatic brain injury are needed to provide the rationale for eventual clinical use.

The present findings emphasize the importance of PAF as a key mediator of secondary brain damage and are supported by an increasing number of reports on the physiological and pathophysiological actions of PAF. This factor is known to exert a variety of effects under variable conditions, especially on the CNS. There are specific binding sites for PAF in the brain with maximum binding in the midbrain and hippocampus. Platelet-activating factor also exerts direct effects on neuronal cells; low concentrations of PAF induced neuronal differentiation in serum-free medium, whereas...
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higher concentrations were found to be cytotoxic. These in vitro effects were mediated by increases in free intracellular calcium. Intracarotid infusion of PAF in the rat was found to decrease cortical CBF by 25%, paralleled by an increase in the cerebral metabolic rate for oxygen, which is a typical phenomenon during the period of delayed postischemic hypoperfusion. This effect could be due to either the direct vasoconstrictive properties of PAF or vasoconstriction mediated by leukotrienes, since granulocytes are sensitive to stimulation by PAF. Furthermore, direct and specific vasoconstrictive effects of PAF on pial arteries in the newborn pig have been described. Intracarotid infusion of PAF in the isolated perfusion rat brain was shown to alter the BBB permeability without itself penetrating it. A recent clinical study provided indirect evidence of augmented PAF generation in ischemic cerebrovascular disorders by demonstrating increased plasma levels of the PAF degrading enzyme acetylhydrolase in stroke patients, which correlated with an elevated PAF-induced platelet activation.

A variety of experimental studies on cerebral ischemia and electroconvulsive disorders support the importance of PAF in neurological injury; PAF inhibition in a model of multifocal cerebral ischemia was found to improve neuronal recovery as assessed by cortical somatosensory evoked potentials in the dog. Spinnewyn, et al., demonstrated beneficial effects not only by preventive but also by curative administration of a PAF antagonist in global cerebral ischemia in the gerbil, showing improved neurological outcome and increased mitochondrial respiration rate. In the same model of cerebral ischemia, PAF inhibition was shown to increase CBF in the phase of delayed hypoperfusion. The latter phenomenon was associated with a decreased free fatty acid pool in the forebrain. This study was in good agreement with the findings of Birkle, et al., who showed decreased accumulation of free fatty acids in mouse brain in postdecapitation ischemia and electroconvulsive shock by inhibition of PAF. No effect, however, was found on the production of diacylglycerol, indicating that the phospholipase A2 might be the major locus of PAF-mediated effects. Furthermore, it was shown that PAF can be produced by brain tissue in electroconvulsive shock and following administration of chemoconvulsant agents in the rat. Tokumura, et al., found evidence for the existence of PAF and various homologues and analogues of PAF in a lipid extract of bovine brain.

Neurotrophils have been suggested to play an important role in the “blood-damaged tissue” interaction in the ischemia reperfusion paradigm. Neutrophil depletion improved neuronal recovery and CBF in multifocal cerebral ischemia by air embolism in dogs and reduced the size of myocardial infarction. In addition, neutrophils were shown to be involved in the postischemic flow derangements which develop after incomplete forebrain ischemia. Our observation of reduced neutrophil accumulation by BN 50739 might be related to direct effects of the PAF, since neutrophils are known to be both a source and a target of PAF. The current study supports the hypothesis that, due to the vasoactive and tissue damaging effects of activated neutrophils, improved neuronal survival in hypoperfusion-related injuries should be associated with improved CBF and reduced neutrophil accumulation. It is beyond the scope of the present study to discuss the exact biochemical and molecular receptor-mediated mechanisms of the actions and effects of PAF, and any speculation in that regard would be premature at this point. However, we have investigated a pathophysiological concept which exclusively focuses on the role of PAF as seen in Fig. 10. We are of course aware that not only one but most likely an array of mediators are involved in these processes, and any schematic concept is at best incomplete.

The Nd:YAG Laser in Neurosurgery

The Nd:YAG laser has proved to be a valuable tool in the hands of neurosurgeons. The present study, however, indicates that, in addition to the primarily ablated tissue block, progressive neuronal cell loss occurs in the vicinity of the lesion, extending the initial lesion diameter by approximately one-third. Furthermore, it appears that the hemostatic properties of the Nd:YAG laser apply to the central lesion core rather than to the boundaries where extravasation of erythrocytes and delayed hemorrhages could be observed within 24 hours after irradiation.

Conclusions

1. The Nd:YAG laser-induced focal brain lesion provides an excellent model of hypoperfusion-dependent brain injury and features typical characteristics of secondary brain damage, such as hypoperfusion, edema,
and BBB disruption, which can be assessed in a highly standardized fashion.

2. Progressive loss of neuronal tissue beyond the site of the primary lesion continues for up to 24 hours after the injury and shows a spatial relationship with impaired microcirculation, edema, BBB disruption, and neutrophil accumulation.

3. Pretreatment with PAF antagonist BN 50739 improved the impaired microcirculation and reduced edema and BBB permeability and neutrophil accumulation.

4. Our study suggests potential beneficial effects of PAF antagonist administration in traumatic brain injury, including improved survival of neuronal cells.

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References


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