The Effect of a Platelet Activating Factor Antagonist (BN 52021) on Neurologic Outcome and Histopathology in a Canine Model of Complete Cerebral Ischemia

Roger E. Hofer, M.D.,* Thomas J. Christopherson, M.D.,† Bernd W. Scheithauer, M.D.,‡ James H. Milde,* William L. Lanier, M.D.§

Background. Research has demonstrated that platelet activating factor may modulate, in part, the severity of postischemic neurologic injury. The proposed mechanism involves a platelet activating factor-mediated release of cerebral cellular lipids and free fatty acids, resulting in increased cerebral edema and cell injury. The present study tested the hypothesis that a specific platelet activating factor antagonist, BN 52021, would improve neurologic outcome after 12 min of complete global cerebral ischemia in a canine model.

Methods. Using an established canine model of complete cerebral ischemia, dogs were assigned randomly to receive, in a blinded fashion, either 20 mg/kg BN 52021 intravenously (N = 8) or placebo (N = 7) 5 min before cerebral ischemia. After cerebral ischemia and recovery, neurologic assessment was performed by a blinded observer for 72 h. Immediately thereafter, the brains were harvested and later were evaluated histologically by a neuropathologist blinded to the treatment groups.

Results. Dogs were well matched for systemic physiologic variables during all portions of the study. One placebo-treated dog and one BN 52021-treated dog were not included in the statistical analysis because of failure to meet preestablished protocol criteria. BN 52021, when compared to placebo, affected neither neurologic functional recovery nor overall histopathology scores. Regional histopathology was improved in BN 52021-treated dogs in only 1 of 18 brain regions studied (Ae., the parietal cortex). When both treatment groups were combined, there was a significant correlation between neurologic function rank and histopathology rank.

Conclusions. The present data demonstrate that the platelet activating factor antagonist BN 52021, at a dose of 20 mg/kg intravenously given 5 min before cerebral ischemia, did not protect the brain from injury in this canine model of complete global ischemia. (Key words: Brain; cerebral ischemia; cerebral protection. Blood: platelet activating factor antagonist; BN 52021; ginkgo B.)

RECENT research investigating the mechanism of ischemic neurologic injury has demonstrated that platelet activating factor (PAF) may contribute to postischemic injury.1 Though the mechanism is not clearly defined, it is hypothesized that PAF may alter postischemic injury by a mechanism that involves the release of cerebral cellular lipids and free fatty acids, resulting in worse cerebral edema and cell injury.2

Based on the above-mentioned hypothesis, additional research has focused on the possibility that platelet activating factor antagonists (PAFAs) may protect the brain from ischemic injury. A specific PFA, BN 52021, an extract from the Chinese tree Ginkgo biloba, has been reported to improve postischemia cerebral energy metabolism in gerbils,3 antagonize cerebral edema induced by microembolization in rats,4 and possess vasodilatory effects on blood vessels during the reperfusion phase of cerebral ischemia.5 Initial studies with BN 52021 indicated that there may be improvement in neurologic functional recovery, as measured by cerebral stroke index, following global ischemia in gerbils.6 The purpose of the proposed study was to (1) evaluate the effect of a specific PFA, BN 52021, on neurologic functional recovery in an established canine model of complete cerebral ischemia, (2) determine whether BN 52021 treatment results in an improvement in postischemic histopathology, and (3) determine whether there is a correlation between postischemic neurologic function injury and histopathology in dogs treated with BN 52021 or placebo.

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Materials and Methods

After Institutional Animal Care and Use Committee approval, the study was completed using 15 purpose-bred dogs weighing 14–18 kg. Anesthesia was induced with 1.5–3.5% halothane inspired in oxygen in an induction box, and once the dogs were anesthetized, the tracheas were intubated and the lungs were mechanically ventilated with a Harvard pump (South Natick, MA). A tidal volume of 15–20 ml/kg was used, and the respiratory rate was adjusted to maintain PaCO2 near 38 mmHg. Anesthesia was maintained with 1.0–2.0% halothane inspired in nitrogen and oxygen during the preparatory period, and the inspired oxygen fraction was adjusted to maintain PaO2 near 150 mmHg. Muscle paralysis was induced and maintained using pancuronium. Esophageal temperature and subtemporalis muscle temperature were measured (model 73A, Yellow Springs Instrument Laboratory, Yellow Springs, OH) and maintained near 37°C with heating pads and lamps. Cannulae were inserted into both femoral arteries using PE120 and PE220 polyethylene catheters. The PE120 catheter was used for continuous blood pressure monitoring; the PE30 catheter was used for blood withdrawal and infusion. Arterial blood gases were determined by electrodes at 37°C (model 1304, Instrumentation Laboratories, Lexington, MA). A cannula also was inserted into a forelimb vein for fluid and drug administration. A three-channel bifrontal, biparietal, and bioccipital electroencephalogram (EEG) was monitored using perioseal needle electrodes and a polygraph (model 8–10, Grass, Quincy, MA). The EEG was used to identify the onset of cerebral ischemia (defined as EEG isoelectricity). Inspired and end-expired oxygen, carbon dioxide, and halothane concentrations were measured using a mass spectrometer (model 1100, Perkin-Elmer, Pomona, CA). All animals received 900,000 units Flo-cillin (procaine-penicillin/benzathine-penicillin; Fort Dodge Laboratories, Fort Dodge, IA) after the induction of anesthesia but before the surgical preparation.

The animals were prepared for the production of complete global cerebral ischemia using a previously described compression technique. Briefly, using Tuohy introducing needles, one polyethylene catheter (PE20) was placed into the lumbar subarachnoid space and two catheters (PE30) were placed into the cisterna magna. The lumbar catheter and one cisternal catheter were used to infuse 37°C saline solution to increase intracranial pressure, and the second cisternal catheter was used to continuously monitor subarachnoid pressure. Halothane was maintained at 0.87% end-expired (1.0 MAC), and arterial blood gases and acid-base status were maintained within protocol limits for a minimum of 20 min before obtaining control physiologic measurements.

Because of previous studies reporting significant differences in neurologic outcome resulting from minor differences in the preischemic blood glucose concentrations,9 blood glucose concentrations were strictly maintained between 95 and 125 mg/dl before control measurements. Thus, if the blood glucose concentration was <95 mg·kg, a 10% glucose infusion was started at a rate of ≤20 ml/h until the blood glucose was within the predetermined range for 20 min. Previous research in a rat model has determined that, by using this low-dose glucose infusion, brain glucose concentrations can be predicted accurately by monitoring blood glucose concentrations.9

After initial control measurements, the dogs were given intravenous treatments of either 20 mg/kg BN 520211 (N = 8), or placebo (BN 52021-carrier consisting of 20 mg mannitol per ml 0.9% NaCl, with pH adjusted to 8.75 with HCl; N = 7). Treatments were administered in a blinded, randomized fashion after the 20-min stabilization period. Repeat measurements of control physiologic variables were recorded every minute for 5 min after drug or placebo bolus administration to determine the possible hemodynamic effects of the study drug. Arterial blood samples were taken 5 min after the injection of either treatment for assessment of changes in arterial blood gases and/or acid-base status.

Thereafter, complete cerebral ischemia was produced using a previously described technique. Briefly, blood was withdrawn from the larger femoral artery cannula into a heparinized reservoir to rapidly reduce the systolic blood pressure to ≤60 mmHg. Upon achieving this target blood pressure, warmed (37°C) saline solution was infused into the lumbar and into one of the cisterna magna subarachnoid conduits until the subarachnoid pressure was at least 20 mmHg greater than systolic blood pressure. This procedure resulted in complete cerebral ischemia, as confirmed by an isoelectric EEG, within seconds. Once the EEG became isoelectric, the inspired halothane and intravenous glucose infusion were discontinued. During this pe-
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The mean arterial blood pressure (MAP) was maintained at 55 ± 5 mmHg, and tendencies toward blood pressure increases during the ischemic period were treated with additional blood withdrawal. After 11 min of ischemia, the MAP was increased to 60–80 mmHg by reinfusion of withdrawn blood. At exactly 12 min of ischemia (determined from the onset of the isoelectric EEG), the subarachnoid conduits were opened to air, resulting in a restoration of cerebral perfusion pressure to >60 mmHg within seconds. During the first 5 min of reperfusion, the MAP was maintained at ≤100 mmHg by blood withdrawal or reinfusion, and the inspired oxygen was increased to 100%. Thereafter, the MAP was maintained at ≤150 mmHg, and the remaining blood was reinfused over the ensuing 15–20 min. When the dogs were judged to be hemodynamically stable, but before the return of EEG activity, they were paralyzed with additional pancuronium to facilitate mechanical ventilation and sedated with 50% N₂O in oxygen in an attempt to prevent cerebral hypermetabolism from the stress of immobilization.# At 1 h posts ischemia, paralysus was reversed with neostigmine (0.07 mg/kg) and glycopyrrolate (0.01 mg/kg), and the nitrous oxide was discontinued. When judged stable, the tracheas were extubated, and the dogs were recovered on a thermostatically controlled heating pad at 37° C for 24 h. They were inspected every 1–2 h for the first 6 h then every 6–8 h thereafter for 72 h. The dogs were returned to their cages after 24 h.

Neurologic assessment was performed by a blinded observer (WLL) at 24, 48, and 72 h posts ischemia. A detailed neurologic scoring scale, modified from D’Alecy et al.,10 was used to evaluate consciousness, motor function, respiration, cranial nerves, and spinal nerves. The 100-point D’Alecy et al. scale assigns points for neurologic deficits. Neurologically intact animals score no points, and brain-dead animals score 100 points. These scores were modified by conversion to per cent function (i.e., a modified D’Alecy et al. score) such that a normal dog received a score of 100 and dead animals were given 0.

After the final observation at 72 h posts ischemia, the dogs were anesthetized with ketamine. After tracheal intubation, the lungs were mechanically ventilated with 100% O₂, and the dogs were paralyzed with pancuronium. A thoracotomy was performed, and in rapid succession, the heart was electrically fibrillated, the descending thoracic aorta was cross-clamped, the left cardiac ventricle was cannulated, and the right atrium was opened. Immediately thereafter, a 4% paraformaldehyde solution was infused at a pressure of 100 cmH₂O to produce in situ brain preservation. The brains were removed 1 h later and stored in buffered paraformaldehyde. All brains were fixed for at least 4 weeks (2 weeks in paraformaldehyde; the remainder in 10% formalin) before gross and microscopic examinations.

Histologic evaluation was performed by a neuropathologist (BWS) blinded to the treatment groups. Coronal whole-mount, paraffin-embedded microsections were cut to a 6-µm thickness and stained with the hematoxylin and eosin method. The type and extent of histopathology was graded according to a previously described scale.11 The severity of injury was assessed bilaterally using a 5-point scale: grade 0 (normal) no ischemic changes; grade 1 (minimal damage) indicating the presence of rare single neurons showing either the eosinophilia typical of ischemic nerve cell change or frank necrosis; grade 2 (moderate damage) <25% of cells damaged; grade 3 (severe damage) 25–50% of cells damaged; and grade 4 (maximal damage) >50% of cells damaged, indicating the presence of ischemic nerve cell change or frank necrosis. The single grade was then multiplied by a weighting factor (infarction, 4×; ischemic nerve cell change, 2×; edema, 1×) to obtain a score for each of 18 brain regions. The analysis for one of these regions, the hippocampus, was subdivided further to provide an assessment of the CA₁, CA₂, and CA₃,₄ segments. The hippocampus was chosen for further analysis because of previous reports of hippocampal sparing by BN 52021 after cerebral ischemia.12 The scores for individual brain regions, as well as a total score for all regions, were statistically compared between treatment groups. A separate analysis was performed to compare injury within the hippocampal segments.

Animals that did not meet all preestablished protocol criteria were excluded from data analysis. Exclusion was based on strict criteria: a preischemic blood glucose >125 mg/ml; evidence of incomplete ischemia; severe cardiopulmonary complications such as pulmonary edema, resulting in a postischemic PaCO₂ <60 mmHg and/or PaO₂ >45 mmHg after extubation; failure to achieve a postischemic MAP of >60 mmHg within 1 min after reperfusion; or a MAP >150 mmHg for more than 1 h posts ischemia. Animals also were ex-

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cluded from statistical analysis if, at the time of brain harvest, there was evidence of gross subpial hemorrhage or interstitial hemorrhage within the brain or brainstem. Previous studies have shown that histologic changes after a period of cerebral ischemia require 24 h or more to mature. Thus, any dog that died within 24 h of the ischemic insult was not included in the data analysis. However, if the dog died after 24 h but before the 72-h observation and data acquisition period, the brain was harvested and preserved at the time of death, and the data from the dog were included in the final statistical analysis.

Physiologic variables were compared using a one-way analysis of variance and unpaired t tests. Functional outcome scores and histopathology scores were compared using Mann-Whitney rank sum tests and the Spearman rank correlation coefficient. \( P < 0.05 \) was considered significant. All data are presented as mean \( \pm SD \).

**Results**

The BN 52021- and placebo-treated groups were well matched for preischemic physiologic variables. There were no significant changes in any of the physiologic variables measured 5 min after the administration of either BN 52021 or placebo (Table 1). All dogs were able to be tracheally extubated after recovery from cerebral ischemia within 2 h after the reversal drugs were given. Two dogs were excluded from statistical analysis for failure to meet the preestablished protocol criteria: one BN 52021-treated dog died within 3 h of the ischemic event, and one placebo-treated dog died within 12 h of the ischemic event. Two other dogs that survived for more than 24 h but less than 72 h were included in the final data analysis. One animal died at 25 h and the other at 27 h. Both were treated with BN 52021. The clinical course and necropsy examination revealed an ischemic neurologic death.

Of the six placebo-treated dogs included in the final data analysis, all sustained functional neurologic deficits varying from ataxia or blindness (modified D’Aley et al. score of 98) to inability to stand (modified D’Aley et al. score of 86) when assessed at 72 h postischemia (Fig. 1). The seven dogs receiving BN 52021 treatment and surviving for more than 24 h were more variable in functional outcome. Scores ranged from no damage (modified D’Aley et al. score of 100) to death within 48 h (modified D’Aley et al. score of 0; Fig. 1). There was, however, no significant difference between the functional outcome scores of the BN 52021- and placebo-treated groups (\( P > 0.9 \)).

BN 52021, when compared to placebo, also did not affect overall histopathology scores (\( P > 0.9 \); Fig. 2). When both treatment groups were combined, regional injury could be assigned to one of three broad classes. In decreasing order, the caudate nucleus, parietal cortex, occipital cortex, frontal cortex, and putamen sustained the greatest injury. This was followed by the temporal cortex, insular cortex, cerebellar Purkinje cells, thalamus, and hippocampus. The third grouping had either no injury or a trace of injury and consisted of the central white matter, corpus callosum, midbrain, globus pallidus, pons, medulla, cerebellar dentate nucleus, and substantia nigra. Of the 18 areas studied,

**Table 1. Physiologic Variables before and 5 Minutes after Administration of Either Placebo or BN 52021 in Dogs**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-placebo Control (n = 6)</th>
<th>5 Min after Placebo</th>
<th>Pre-BN 52021 Control (n = 7)</th>
<th>5 Min after BN 52021</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{ao} ) (mmHg)</td>
<td>154 ± 6</td>
<td>155 ± 4</td>
<td>150 ± 12</td>
<td>155 ± 24</td>
</tr>
<tr>
<td>( P_{aco} ) (mmHg)</td>
<td>37 ± 2</td>
<td>41 ± 1</td>
<td>38 ± 1</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.04</td>
<td>7.34 ± 0.02</td>
<td>7.34 ± 0.03</td>
<td>7.32 ± 0.03</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>92 ± 14</td>
<td>93 ± 17</td>
<td>96 ± 10</td>
<td>97 ± 19</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>128 ± 22</td>
<td>138 ± 20</td>
<td>121 ± 27</td>
<td>121 ± 29</td>
</tr>
<tr>
<td>Core temperature (°C)</td>
<td>37.0 ± 0.0</td>
<td>37.0 ± 0.0</td>
<td>37.0 ± 0.0</td>
<td>37.0 ± 0.0</td>
</tr>
<tr>
<td>Cranial temperature (°C)</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>36.9 ± 0.2</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>111 ± 11</td>
<td>114 ± 11</td>
<td>114 ± 7</td>
<td>121 ± 15</td>
</tr>
</tbody>
</table>

Data are mean ± SD. \( P_{ao}, P_{aco} \), and blood glucose are from arterial samples. 
MAP = mean arterial blood pressure; Core temperature = esophageal temperature; Cranial temperature = temperature beneath the temporalis muscle; HR = heart rate.

* There were no significant differences with time or between groups.

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Fig. 1. Rank of neurologic function in BN 52021- and placebo-treated dogs at 72 h postischemia. Neurologic function scores were calculated using a modification of the scoring scale of D'Alcey et al. Percent function ranged from 100 (normal) to 0 (dead), and these scores were ranked from 1 (best outcome) to 13 (worst outcome). There was no significant difference in neurologic function between groups ($P > 0.9$).

Fig. 3. Correlation between neurologic function rank (using a modified D'Alcey et al. scoring scale) and histopathology rank (from best [1] to worst [13]) in dogs treated with either BN 52021 or placebo. The solid line represents the line of identity ($r = 0.67; P < 0.02$).

Fig. 2. Rank of total histopathology scores in BN 52021- and placebo-treated dogs at 72 h postischemia. Scores ranged from 12.0 to 37.5 points and were ranked from 1 (best outcome) to 13 (worst outcome). There were no significant differences between groups ($P > 0.9$).

However, differences between the BN 52021- and placebo-treated groups approached statistical significance only in the CA3,4 segment ($P = 0.06$). When data from both treatment groups were combined, of the original 18 brain areas analyzed, 5 had histopathologic injury greater than in any hippocampal segment.

There was a good correlation between overall histopathology score ranks and functional outcome ranks ($r = 0.67; P < 0.02$; fig. 3). When the relationship between hippocampal segment histopathology score ranks and functional outcome ranks was compared, the relationship achieved statistical significance for the CA3 segment ($r = 0.54; P < 0.05$). There was no statistical significance when comparing the CA1 histopathology segment rank or the CA3,4 histopathology segment rank with the modified D'Alcey et al. functional score rank ($r = 0.47; P = 0.07; r = 0.27; P = 0.32$, respectively).

Discussion

Many theories of cellular injury mechanisms and their relationship to tissue ischemia are being investigated. A potentially crucial mediator of diverse pathologic processes, PAF, was first demonstrated in the early 1970s, and it now appears that it may have a role in determining the severity of posts ischemic neurologic injury. PAF is a naturally occurring ether phospholipid generated by specific activation of basophils, poly-

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morphismuclear leukocytes, macrophages, and eosinophils during anaphylaxis, shock, or ischemia. The role of PAF has been identified only recently as contributing to many different pathologic processes, including central nervous system disorders (e.g., Zellweger syndrome, panic disorders, trauma- and ischemia-induced brain injury). 1,15

Recently, it was demonstrated that PAF may contribute to the exacerbation of ischemic cellular damage and promote brain edema. 6 PAF has been detected in large amounts in the Mongolian gerbil brain after complete cerebral ischemia and reperfusion. 1 Furthermore, specific binding sites for [3H]-PAF have been demonstrated in both gerbil and rat brains, 1 and these binding sites have been linked to an increase in intracellular calcium, activation of phospholipases, and activation of other secondary messenger systems. 16

PAF can be antagonized directly or indirectly by a variety of drugs. 15 A new category of drugs, the PAFAs, are being studied to determine whether their use will improve neurologic function after cerebral ischemia. BN 52021, a specific PAFa from a standardized extract of the Chinese tree Ginkgo biloba, has been shown to be the portion of the extract with the most potent activity antagonizing the PAF effect on the brain. 6 Although the mechanism of action is still undefined, some authors have concluded that PAFAs act primarily by attenuating the secondary neurologic injuries that occur during the postischemic reperfusion phase. 17

Studies using rats have reported that pretreatment with a ginkgoiide extract containing BN 52021 reduced experimental cerebral edema induced with intraperitoneal triethyltin. 18 Preservation of cerebral energy metabolism and increasing survival time in rats exposed to hypoxic hypoxia after intraperitoneal pretreatment with BN 52021 has been reported. 18 Fitzpatrick et al. 19 using an isolated dog brain preparation, reported that postischemia BN 52021 treatment resulted in increased concentrations of cortical ATP and phosphocreatine, improved cerebral vascular resistance, and improved postsischemic functional recovery as assessed by an increased amplitude of the brain auditory evoked potentials. Thus, BN 52021 would appear to have beneficial effects in rodent animal models and the isolated dog brain preparation.

Presently, the only study that used an intact animal model of complete global ischemia to evaluate the effect of BN 52021 on outcome is from Spinnewyn et al. 17 These authors used an established postischemic scoring system (cerebral stroke index) in gerbils. Although they demonstrated a BN 52021-associated improvement in behavior patterns following transient ischemia, the study evaluated neurologic function for only 6 h postischemia and did not address histopathology. In the present study, the dogs were followed for 72 h postischemia, in contrast to shorter periods (i.e., 6 h or less) in the above-mentioned studies. It is known that, following an ischemic insult, histologic injury continues to mature for 2–3 days. 20 Thus, it is possible that the magnitude of benefit described in prior studies may have been sufficient to alter short-term anatomy and physiology but insufficient to alter long-term outcome.

More recently, it has been reported that BN 52021 reduced postischemic histologic damage of the hippocampal CA1 area in rats pretreated (1 h before cerebral ischemia) with oral BN 52021 and in gerbils treated before (oral) and after (intraperitoneal) cerebral ischemia. 12,17 In the present canine study, BN 52021 did not beneficially affect hippocampal histologic injury but may have augmented injury in the hippocampus, specifically in the CA3,4 areas. Furthermore, when 18 brain regions were examined, we found evidence of a beneficial BN 52021-induced effect in only 1, the parietal cortex. We specifically did not observe protection in the hippocampus, as has been reported previously. 12,17 These patterns of results may be due to species difference, as previously discussed. 21

Possible reasons for our negative results might include: (1) the degree of ischemia was too small to show an effect of BN 52021, or the model was not sensitive enough to provide evidence of a treatment effect. This is unlikely because a previous study employing this model found a significant beneficial effect produced by the antioxidant U74006F; (2) the dose was not appropriate for this model. The dose administered to this canine model of cerebral ischemia was calculated based on known pharmacokinetics of BN 52021 in dogs and assured that each dog receiving BN 52021 had therapeutic blood levels for about 24 h; and (3) the time of administration was not appropriate. Previous studies have shown that, in rodent models, BN 52021 was protective if administered before, during, or after cerebral ischemia. 5,12

In the present study, we offer the first report of the
effect of BN 52021 on both long-term functional neurologic recovery and histopathology in an established animal model of complete cerebral ischemia. In our model, pretreatment with BN 52021 20 mg/kg did not protect the brain from either functional or histologic ischemic injury. There was an excellent correlation between functional outcome and global histopathology using this model, suggesting that the model functioned appropriately.

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References


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