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Effects of Buprenorphine on Intracerebral Collagenase-induced Hematoma in Sprague-Dawley Rats

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We evaluated the effects of buprenorphine (0.05 mg/kg intraperitoneally) after collagenase-induced intracerebral hemorrhage in Sprague-Dawley rats. Methods of evaluation included serum biochemistry, behavioral tests (neurologic exam and rotarod treadmill), and histopathology. Serum biochemistry parameters showed no change after surgery in controls and buprenorphine-treated animals. At 48 h after collagenase injections, the performance of treated rats on the rotarod treadmill test was not significantly different from that of untreated rats, but the neurologic exams of treated rats showed significantly improved performance. Although the volume of the hematoma was reduced with buprenorphine, the number of necrotic neurons in the penumbra was significantly increased. These data indicate that administration of buprenorphine led to neurologic and histopathologic differences in a rat model of intracerebral hemorrhage, and data from such studies should be interpreted carefully if an opioid analgesic is used to minimize pain.

Animal models play an important role in the study of mechanisms and treatments of traumatic brain injury.[6] These models have been useful for the study of neuropathologic processes associated with edema and hemorrhage. Recent studies have evaluated the use of corticosteroid therapy with drugs commonly used in veterinary medicine[10,11,20] by using a hematoma model developed by Rosenberg and colleagues,[18] in which collagenase is injected sterotaxically into the caudoputamen nucleus. This model reproduces many characteristics of cerebral hemorrhagic processes of traumatic brain injury seen in mammals and humans. The size of the hematoma has been shown to be reproducible, and histopathology is similar to spontaneous intracerebral hemorrhage.[5] However, the evaluation of drugs for the treatment of this condition can be influenced by drug interactions and possible interference with the inflammatory process, thereby altering the study conclusions.

Analgesic drugs used in small rodents are mainly opioids and nonsteroidal anti-inflammatory drugs.[7] Opioid analgesics are known to have anti-inflammatory properties.[1,4,9,17] Mu opioid receptor-activating drugs such as morphine decrease edema, hyperalgesia, and pain in animal models of inflammation. In addition kappa opioid-receptor activating drugs may have anti-inflammatory properties.[20] Administration of buprenorphine (partial mu agonist) to relieve pain after surgery therefore could interfere with the evaluation of anti-inflammatory drugs tested for the treatment of intracerebral hemorrhage. The objective of this study therefore was to evaluate the effects of buprenorphine on a collagenase-induced intracerebral hemorrhage in rats by evaluating blood biochemistry, motor behaviors, brain edema and histopathology.

Material and Methods

Animals. We used 32 male Sprague-Dawley rats weighing between 300 to 350 g in this study. These rats were specific pathogen-free and originated from a barrier facility (Charles River, St-Constant, Quebec, Canada). After their arrival, they were kept in a standard laboratory animal environment (fresh filtered air, 15 changes/h; temperature, 21 ± 3 °C; humidity, 40% to 60%; and light-dark cycle, 12:12-h). The rats were housed in polycarbonate cages (Ancare, Bellmore, NY) on hardwood bedding (Beta Chips, Northeastern Products, Warrenburg, NY) and acclimated for at least 5 d prior to initiation of the study. Rats received tap water and a certified laboratory diet (Mouse/Rat Diet, Harlan Teklad, Bartonville, IL) ad libitum. The experimental protocol was approved by the Faculty of Veterinary Medicine Institutional Animal Care and Use Committee prior to animal use, in accordance with the guidelines of the Canadian Council on Animal Care.[2]

Surgical procedure. After isoflurane induction, rats were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA) equipped with an isoflurane adaptor, and surgeries were performed under isoflurane anesthesia. Rectal temperature was monitored (Thermalert TH-8, Physitemp, Clifton, NJ) and kept within normal limits (36 to 37.5 °C) by use of an electric heating pad. A sagittal skin incision was made from between the eyes to the occipital crest. The periosteum was scrapped laterally, and a burr hole (diameter, 1.5 mm) was drilled in the bone at 0.0 mm antero-posterior and 3.0 mm lateral to bregma.[16] The injection site was in the caudoputamen nuclei of the right hemisphere at 5.5 mm from the dura. Collagenase solution (2 µl; 0.5 U Collagenase Type VII, Sigma-Aldrich, Oakville, ON, Canada) was injected over a period of 10 min (0.2 µ/min) by use of a 5-µl Hamilton syringe. The needle was removed 5 min after the end of the injection, to prevent reflux. The skin was sutured with 2-0 silk. Postoperatively, animals received 2 ml of saline subcutaneously and were kept under a heating lamp until recovery.

Treatments. Controls (n = 16) were operated animals that received intracerebral injection of collagenase only. The treatment group (n = 16) received buprenorphine (0.05 mg/kg intraperitoneally) immediately after surgery. A buprenorphine solution (1:9 drug:saline) was prepared from Buprenex (Reckitt Benckiser Pharmaceuticals, Richmond, VA) and vigorously agitated prior to administration. Each group was subdivided into 2 subgroups (n = 8), the first to evaluate brain water content and blood

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biochemistry and the second to evaluate motor performance by neurologic exam and the rotarod treadmill test as well for identification of morphometric cellular changes. All behavioral and morphometric analyses were performed blinded.

Blood biochemistry. Blood (0.5 ml) from the jugular vein of each rat was collected in 1-ml heparinized tubes (Becton Dickenson, Franklin Lakes, NJ) immediately prior to and 24 h after surgery. The following parameters were evaluated automatically (Synchron CX5 Clinical System, Beckman Coulter, Fullerton, CA): glucose, total protein, urea nitrogen, creatinine, albumin, globulin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, Na, K, Cl, Mg, Ca, and PO_4. Experimental values were compared with published findings.[12]

Behavioral evaluations. A semiquantitative neurologic exam was used to evaluate the motor performance of the rats prior to and at 24 and 48 h after surgery. All animals were trained for 3 consecutive days prior to the baseline neurologic exam. This exam has been described previously.[10] The behavioral evaluation, scored between 0 (poor or absent) and 4 (normal performance), comprised the following tests: activity, locomotion, positional passivity, visual positioning, climbing, tail rigidity, tremor, and hopping.

Animals also were tested on a rotarod treadmill (ENV-576, Med Associates, St Albans, VT). After being trained, rats were evaluated prior to and at 24 and 48 h after surgery by setting the rotarod to the acceleration mode of 3 to 30 revolutions per min over 5 min. The maximal time the rat stayed on the rotarod, to a total time of 5 min, was recorded for each performance. A mean of 3 trials was used to represent animal performance. Values are reported as a percentage of the baseline evaluation.

Determination of brain water content. At 48 h after intracerebral injection, animals were injected with pentobarbital (100 mg/kg intraperitoneally) to induce anesthesia overdose, and brains were removed quickly. Hemispheres were separated and cut into 4 coronal sections of approximately equal thickness in the anteroposterior direction and identified as the frontal, P1 (anterior parietal), P2 (posterior parietal), and occipital regions of the right and left cerebral hemispheres. Each slice was placed in a glass tube and was weighed before and after drying for 24 h in an oven at 100 °C. Brain water content was expressed as the percentage change between wet weight and dry weight according to the following formula:

$$(\text{Wet weight} - \text{dry weight})/\text{wet weight} \times 100\%$$

Morphometric and cell count evaluations. At 48 h after intracerebral injection, animals (n = 24) were perfused through the heart under deep anesthesia (pentobarbital, 100 mg/kg intraperitoneally), first with a physiologic dextrose-sucrose solution (200 ml/rat; solution composition (1 l): 8 g NaCl, 4 g dextrose, 8 g sucrose, 0.23 g $CaCl_2$ [Sigma, St Louis, MO]) and then followed by 10% buffered formalin (200 ml/rat) solution. For microscopic evaluation, brains were fixed in formalin for 48 h, embedded in paraffin, and sectioned at 5 µm. Sections were stained by use of a standard cresyl violet method. Hematoma volume was calculated by multiplying the hematoma surface area by the distance between 2 consecutive brain sections (100 µm). In addition, the numbers of neutrophils in the hemorrhagic area and necrotic neurons in the penumbra were evaluated. Because of the difficulty in selecting common histopathologic characteristics of necrotic neurons, only cells that showed typical cytoplasmic shrinkage were evaluated. Evaluation of hematoma volume and cell counts (average of 4 fields at ×20) were performed with an image analysis system (Simple PCI, Compix Imaging Systems, Cranberry Township, PA).

Statistical analysis. The threshold for statistical significance was set a priori at $P = 0.05$. Analysis of variance and post hoc Tukey tests were performed with SAS (version 8.02, SAS Institute, Cary, NC). For analysis of variance, a linear model with repeated measures using time as the intersubject factor and treatment as the intrasubject factor was used. Standard errors were calculated for all reported mean values except for biochemistry results. A simple *t* test was performed for blood biochemistry parameters.

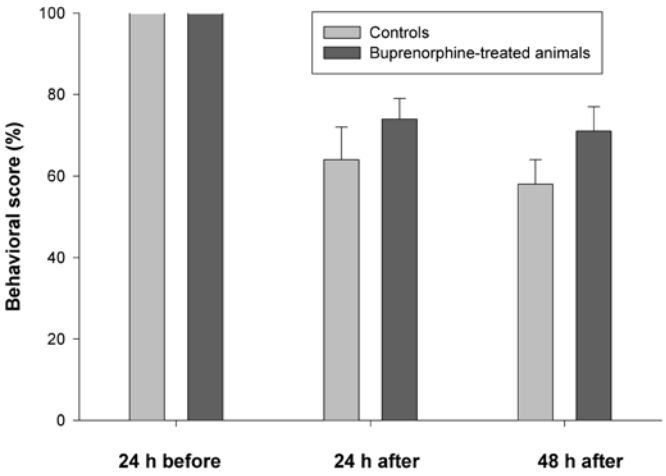

Figure 1. Neurologic scores (mean ± standard error) 24 and 48 h after surgery in Sprague-Dawley rats (6 per group) as a percentage of baseline scores 24 h before intracerebral injection of collagenase. The scores of treated and nontreated animals differed significantly ($P < 0.01$) at 48 h after intracerebral injection of collagenase.

Results

Blood biochemistry. No statistical differences between control and buprenorphine-treated animals were noted for any of the parameters of blood collected at 24 h after surgery. All values were within normal limits, except glycemia decreased after surgery in controls (presurgery versus postsurgery, 11.6 ± 1.85 versus 9.8 ± 2.22 mmol/l) and buprenorphine-treated rats (11.9 ± 2.4 mmol/l versus 9.3 ± 0.58 mmol/l, $P < 0.02$); alkaline phosphatase decreased after surgery in controls (364.9 ± 56.4 versus 259.2 ± 50.3 U/l, $P < 0.01$) and buprenorphine-treated animals (303.1 ± 88.7 versus 198.7 ± 82.3 U/l, $P < 0.05$); and phosphorus decreased after surgery in controls (2.7 ± 0.4 mmol/l versus 2.2 ± 0.3 mmol/l, $P < 0.01$) and buprenorphine-treated animals (2.5 ± 0.2 versus 2.1 ± 0.2 mmol/l, $P < 0.01$). Animals were weighed prior to and after surgery. At 24 and 48 h, 7% and 2% weight losses were observed for both controls and treated animals. This weight loss might explain the decreased glycemia and alkaline phosphatase values as being associated with fasting.

Behavioral evaluations. Behavioral scores for the neurologic exam (mean ± standard error) are presented in Figure 1. The semiquantitative neurologic exam revealed a beneficial effect of treatment ($P < 0.0001$), an effect with time ($P < 0.0001$), and an interaction between time and treatment ($P < 0.0001$). At 48 h, buprenorphine had a net beneficial effect (buprenorphine versus control, 71% versus 58%; $P < 0.01$) as seen with the neurologic exam. No statistically significant effect of treatment was noted with the rotarod treadmill test (Figure 2). In previously reported results,[10,11] sham-operated animals demonstrated normal neurologic exams at 24 and 48 h after collagenase injection.

Brain water content. Brain water content did not differ between controls and rats treated with buprenorphine (Figure 3). In all animals, the anterior parietal slices (P1) of the right hemisphere, which corresponds to the area where the hematoma is localized, had significantly ($P < 0.0001$) increased brain water content.

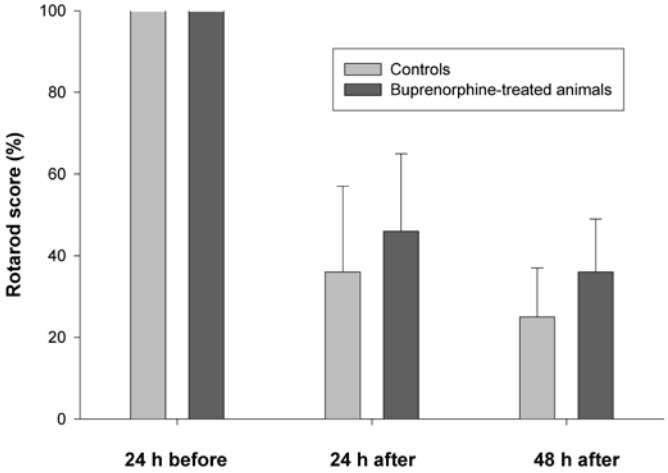

Figure 2. Rotarod treadmill scores (mean ± standard error) 24 and 48 h after surgery in Sprague-Dawley rats (6 per group) that received intracerebral collagenase with or without buprenorphine (0.05 mg/kg intraperitoneally). No significant differences were noted between controls and buprenorphine-treated animals.

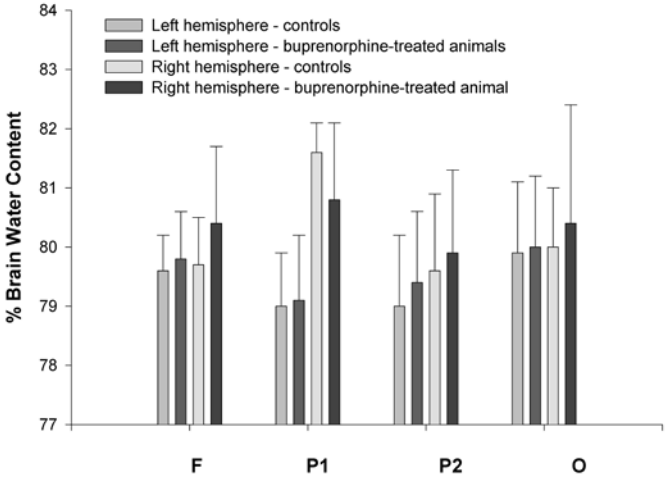

Figure 3. Percentage brain water content (mean ± standard error) 48 h after surgery in Sprague-Dawley rats (6 per group) that received intracerebral collagenase with and without buprenorphine (0.05 mg/kg intraperitoneally). Water content was determined in 4 coronal sections (frontal [F], anterior parietal [P1], posterior parietal [P2], and occipital [O]) of approximately equal thickness taken from the right and left cerebral hemispheres.

No significant differences were noted between controls and buprenorphine-treated animals.

Morphometric and cell number changes. The hematoma volume was significantly ($P < 0.01$) smaller in rats treated with 0.05 mg/kg buprenorphine (21.9 ± 5.5 mm^3) than in control animals (26.3 ± 6.1 mm^3). Neutrophils were observed mainly at the periphery of the lesion, and their numbers did not vary between controls and treated animals. Buprenorphine-treated animals had more necrotic neurons (151 ± 21) than did controls (90 ± 17; $P < 0.0001$). Figure 4 shows the number of necrotic neurons in 6 frontal sections 100 µm apart from each other and taken at the center of the hematoma lesion for all animals. The buprenorphine-treated animals had greater numbers of necrotic neurons at all levels of the lesions.

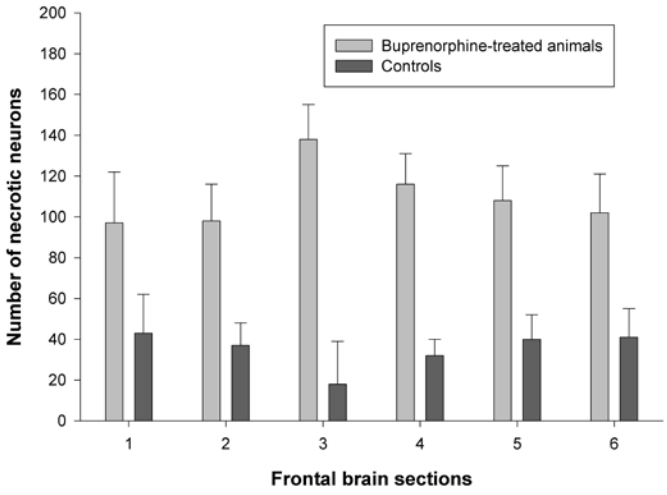

Figure 4. Number of necrotic neurons (mean ± standard error) in 6 frontal sections (thickness, 5 µm) 100 µm apart at the center of the hematoma lesion for rats (n = 6 per group) with and without buprenorphine (0.05 mg/kg intraperitoneally).

Discussion

When compared with those of controls, the neurologic exam suggests that buprenorphine administered at a dose of 0.05 mg/kg intraperitoneally caused a notable change. Histopathologic differences also were apparent. A decrease in the hematoma volume and an increase in the number of necrotic neurons in the penumbral area were seen with buprenorphine treatment, but brain water content did not differ. These findings suggest that the decreased hematoma volume was mainly responsible for the neurologic differences seen in buprenorphine-treated animals. Recent findings have suggested that deterioration of motor capacities with time may be related to the progressive increase of edema and necrosis observed in cerebral tissue.[3] Although neuronal cell death was more extensive in the penumbral area of buprenorphine-treated animals, the neurologic results were less severe than expected from histopathologic findings due to smaller hematoma volume size seen in treated animals.

Because of an obvious breakdown of the blood-brain barrier after intracerebral hemorrhage, a greater concentration of circulating drugs will invade local brain tissue. This effect could be a reason for the greater neuronal cell death in buprenorphine-treated rats. This increase in neuronal death was not observed with corticosteroid treatments.[10,11] Other physiologic parameters known to influence brain damage are body temperature and glycemia. Hypothermia reduces edema, blood-brain barrier disruption, and inflammation, leading to cell necrosis but has no effect on hematoma volume.[13] Hyperglycemia also leads to more severe brain damage when compared to brain damage in normoglycemic animals.[8] In our experiments, body temperature was maintained within normal limits and animals were normoglycemic after surgery, suggesting that these factors did not interfere with the interpretation of our results.

The histologic features of the hematoma at 48 h after injection of collagenase have been described previously.[18] The hematoma zone consisted of cellular debris, neutrophils, scattered clusters of erythrocytes, and necrotic parenchyma. No macrophages were observed. Clear spaces within the nervous tissue, interpreted as edema, surrounded the penumbral region and extended along the corpus callosum in the ipsilateral and contralateral hemispheres. Our results show that neutrophil counts within the hematoma were not significantly different between controls and buprenorphine-treated animals. Neutrophils have been associated with ischemic damage in the brain.[14,15,19] A direct correlation has been established between neutrophil infiltration and ischemic brain injury.[14] Enhancement of intercellular adhesion molecules, inducing endothelial adhesion and transmigration of neutrophils, occurs with ischemia.[15] Therefore the reduced

inflammation with buprenorphine cannot be correlated with the local decrease of neutrophils.

In conclusion, neurological and histopathologic changes occur in association with administration of buprenorphine in a rat model of intracerebral hemorrhage. Therefore care should be taken in the interpretation of results of studies designed to evaluate anti-inflammatory drugs if an opioid analgesic is used to minimize pain.

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