Collagenase-Induced Intracerebral Hemorrhage in Rats

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Intracranial bleeding is an important cause of brain masses and edema. To study the pathophysiology of intracerebral hemorrhage, we produced experimental hemorrhages in 53 rats and characterized the lesion by histology, brain water content, and behavior. Adult rats had 2 μl saline containing 0.5 unit bacterial collagenase infused into the left caudate nucleus. Histologically, erythrocytes were seen around blood vessels at the needle puncture site within the first hour. By 4 hours there were hematomas, the size of which depended on the amount of collagenase injected. Necrotic masses containing fluid, blood cells, and fibrin were seen at 24 hours. Lipid-filled macrophages were observed at 7 days and cysts at 3 weeks. Water content was significantly increased 4, 24, and 48 hours after infusion at the needle puncture site and for 24 hours in posterior brain sections. Behavioral abnormalities were present for 48 hours, with recovery of function occurring during the first week. Brain tissue contains Type IV collagen in the basal lamina. Collagenase, which occurs in an inactive form in cells, is released and activated during injury, leading to disruption of the extracellular matrix. Collagenase-induced intracerebral hemorrhage is a reproducible animal model for the study of the effects of the hematoma and brain edema. (Stroke 1990;21:801-807)

Intracerebral hemorrhage occurs frequently in patients with cerebrovascular disease, brain tumors, or head trauma. The effects of a hematoma on brain tissue have been studied experimentally using the direct infusion of blood and by inducing a hemorrhage after infarction. During a study of the effect of proteolytic enzymes on the extracellular matrix in rats, we observed that the metalloproteinase collagenase injected directly into the caudate nucleus caused an intracerebral hematoma. Earlier investigators had shown an increase in blood-brain barrier permeability following intraventricular injection of this enzyme. Collagenases are proteolytic enzymes that are present within cells in an inactive form and that are secreted at sites of inflammation by mononuclear cells and by metastatic tumors. Brain tissue contains collagen in the basal lamina of blood vessels. We describe the effects of collagenase-induced intracranial bleeding on histology, brain water content, and behavior over 3 weeks.

Materials and Methods

We anesthetized 53 adult male Sprague-Dawley rats weighing 200–300 g with 50 mg/kg i.p. pentobarbital. Rats were placed in a stereotactic apparatus (David Kopf Instruments, Tujunga, California), and a 23-gauge needle was implanted into the caudate nucleus at coordinates (A5.8, L3.0, H1.0). The 40 experimental rats had 2 μl saline containing 0.01–1 unit bacterial collagenase (Type XI or Type VII, Sigma Chemical Co., St. Louis, Missouri) infused by a microinfusion pump (Harvard Apparatus, South Natick, Massachusetts) over 9 minutes. Initially, Type XI collagenase with some protease contamination was used. Subsequent studies were done with Type VII collagenase that was essentially free of proteases. The 13 control rats had similar infusions of 2 μl saline. After infusion, the needle was removed and the wound was sutured. The rats recovered from surgery in a warm place with access to food. They were killed by the intracardiac injection of KCl. The experimental protocols were approved by the Animal Research Committee and adhered to National Institutes of Health guidelines.

For histology, nine experimental rats were infused with 0.01 (n=3), 0.1 (n=2), or 1 (n=4) unit (1,680 units/mg) Type XI collagenase; 4 hours later they were anesthetized again and killed. The brains were...
removed, placed in phosphate-buffered 10% formalin for 24 hours, and embedded in paraffin for sectioning on a microtome into 4-μm slices. Slides were stained with hematoxylin and eosin, cresyl violet (Nissl), or Luxol fast blue. Four control rats were infused with saline, two with heat-inactivated collagenase, and two with 250 units hyaluronidase (Sigma) and killed 4 hours later to test for nonspecific effects of the infusion protocol. Three experimental rats infused with 0.5 unit Type VII collagenase were killed 10, 20, or 45 minutes later to determine the early histologic changes caused by collagenase. Six experimental rats infused with 0.5 unit Type VII collagenase were killed 7 days or 3 weeks later to determine late histologic changes.

For the determination of brain water content, 13 experimental and control rats were infused with 0 (n=2), 0.1 (n=3), 0.5 (n=5), or 1 (n=3) unit Type XI collagenase; 4 hours later they were killed, the brains were removed and divided into hemispheres, and each hemisphere was sectioned into four parts from front to back. Each section was weighed before and after drying for 24 hours in an oven at 100°C. Water content was expressed as the percentage change between wet weight (WW) and dry weight (DW): \( \frac{(WW - DW) \times 100}{WW} \). Another six experimental rats had 0.5 (n=3) or 1.0 (n=3) unit Type XI collagenase infused to study the effect on brain water after 24 hours. Thirteen experimental rats were infused with 0.5 unit (1,920 units/mg) Type VII collagenase and killed 4, 24, or 48 hours later. The brains were prepared as above. These 13 rats were compared with five control rats injected with saline and killed 4 hours later.

A behavioral rating scale to grade the extent of injury was modified from published scales. Three behaviors were tested: ipsilateral circling, bilateral grasp, and beam walking. The extent of circling to the side of the infusion was graded from 0 (no circling) to 4 (always circled). Grasp was tested by placing the rat's paws on the edge of a box 14 inches high; strength of the hemiparetic paw was graded from 0 (grasped well) to 4 (unable to grasp with forepaw). Beam walking was graded by placing each rat on a beam; a grade of 0 indicated that it easily traversed the beam, while a grade of 4 was given those unable to walk on the beam. A total injury score was calculated as the sum of the grades on the three tests.

Data were analyzed using SAS. Statistical significance \( (p<0.05) \) was determined using one-way analysis of variance with the Bonferroni correction for multiple tests.

**Results**

Infusion of 2 μl saline into the caudate nucleus caused a small focal area of damage around the needle in the control rats (Figure 1). Rats infused with heat-inactivated collagenase or hyaluronidase showed needle damage similar to controls. Infusion of 0.01 unit collagenase resulted in a lesion slightly larger than that in controls, and increasingly larger lesions were produced by infusions of 0.1 and 1 unit collagenase. The extent of the lesion depended on the amount of enzyme injected. Bleeding was seen 10 minutes after infusion around a large, thin-walled vessel in the caudate nucleus (Figure 2, top). Rats studied 20 and 45 minutes after infusion had intact erythrocytes dissecting between normal brain cells in the caudate nucleus (Figure 2, bottom). By 4 hours there was extensive bleeding without evidence of tissue necrosis. Erythrocytes dissected into the tissue, and brain cells remained intact.

At 24 hours after infusion the lesion consisted of two zones. The large central zone had a mosaic-like appearance, with numerous areas composed of pale-
staining, extravasated erythrocytes separated by fragments of necrotic parenchyma. The sharp border between these two elements was in places obscured by extravasated fibrin found near necrotic blood vessels containing fibrin thrombi. The peripheral zone of the lesion consisted of a narrow band of poorly staining parenchyma containing either viable or necrotic glial and neuronal cells. There were foci of hemorrhage composed of well-preserved erythrocytes and occasional polymorphonuclear leukocytes. This zone also contained intact blood vessels and some with necrosis and fibrin thrombi within their lumina. After 48 hours, the changes were similar to those observed at 24 hours (Figure 3, left), except that in the central zone erythrocytes were in a more advanced stage of decay, while the peripheral zone contained an intense infiltrate of polymorphonuclear leukocytes (Figure 3, right). The peripheral zone contained extravasates of preserved erythrocytes, blood vessels with hypertrophied endothelium, and sparse mononuclear cells in the perivascular spaces.

Seven days after infusion, the mosaic-like pattern was still seen in the central zone, where fragments of the necrotic parenchyma were recognizable only by the presence of ghost cells (Figure 4). The erythrocytes were extremely pale and often had ill-defined contours. There were also rare macrophages and small vascular profiles with swollen endothelium, mostly at the edges of the central zone. The peripheral zone consisted of a broad band of densely packed macrophages, many containing multiple lipid droplets. A few macrophages containing hemosiderin
Figure 3. Photomicrographs of histologic changes in caudate nucleus of rat 48 hours after infusion of 0.5 unit collagenase. Left: Large lesion protrudes into lateral ventricle. Central white matter of each hemisphere behind basal ganglia exhibits some pallor. Right: Infiltrate of polymorphonuclear leukocytes at periphery of lesion (hematoxylin and eosin stain, X229).

FIGURE 4. Photomicrograph of histologic changes in caudate nucleus of rat 7 days after infusion of 0.5 unit collagenase. Band of macrophages surrounds central zone, which exhibits mosaic pattern (hematoxylin and eosin stain, X36).

were found at the outer limits of the peripheral zone. In places, small fragments of necrotic parenchyma were discernable among the macrophages. There was an increase in vascularity, with small blood vessels characterized by plump endothelium.

Twenty-one days after infusion, the two zones were no longer apparent. The lesion was transformed into a cyst traversed by delicate gliovascular trabeculae, the meshes of which contained variable numbers of hemosiderin and lipid-laden macrophages. At the border of the cyst there were variable numbers of reactive astrocytes.

Edema present 4 hours after infusion depended on the amount of collagenase infused (Figure 5). Water content at the needle puncture site was significantly greater than that in the contralateral hemisphere of rats infused with 0.5 or 1.0 unit collagenase (p<0.05). No rat injected with 1.0 unit collagenase survived for 24 hours, while those given 0.5 unit did. Therefore, 0.5 unit was selected for long-term experiments.

Water contents in the four sections of each hemisphere 4, 24, and 48 hours after the infusion of 0.5 unit collagenase are shown in Figure 6. By 4 hours a significant increase was seen at the needle puncture.
site and in both posterior sections. Rats killed at 24 hours had significantly increased water contents in a similar pattern, except the contralateral caudate nucleus was also edematous. At 48 hours water content was increased only in the infused hemisphere.

Total injury scores over 3 weeks are shown in Figure 7. Before infusion the rats performed all tests without difficulty, as shown by the score of 0. Some variability, most likely related to the anesthetic, was seen at 4 hours. Performance remained significantly impaired for up to 48 hours. Recovery began by 72 hours and continued for the 3 weeks of testing, with most of the improvement seen during the first week.

Discussion

We found that an intracerebral hemorrhage was produced by the infusion of bacterial collagenase into the caudate nucleus. Ten minutes after injection, erythrocytes were seen around large caudate blood vessels. There was extensive bleeding 4 hours after the infusion of ≥0.5 unit collagenase, with tissue disruption by the dissecting erythrocytes. Brain edema and behavioral abnormalities began to resolve by 48 hours. Histologically, collagenase caused a hemorrhagic lesion, with erythrocytes seen in the perivascular spaces and in white matter fiber tracts. Consolidation of the necrotic mass resulted in a cyst by 3 weeks. There was diffuse brain edema, maximal at the needle puncture site, but also present in both posterior brain sections and in the contralateral caudate nucleus.

Earlier investigators, who simulated an intracerebral hemorrhage by injecting 0.5–1.5 ml blood into the brains of dogs, observed dissection of tissue, with slit-like lesions through the white matter. More recently, investigators showed that the intracerebral injection of 0.05–0.25 ml autologous blood into the brains of cats reduced cerebral blood flow in the tissue around the hematoma. Hemorrhages have been observed to occur sporadically after experimental infarction.

We found that rats injected with ≥0.5 unit bacterial collagenase had extensive edema at multiple

![Figure 5. Bar graph of effect of different doses of collagenase in 2 μl saline on water content in contralateral (open bars) and ipsilateral (shaded bars) caudate nucleus of 13 rats 4 hours after infusion. *p<0.05 different from contralateral by analysis of variance.](image)

![Figure 6. Bar graph of water content in four brain sections of 13 rats 4, 24, and 48 hours after infusion of 0.5 unit collagenase into caudate nucleus. Small drawing in center shown regions sampled and needle puncture site. *p<0.05 different from control by analysis of variance.](image)
sites. All rats injected with 1 unit collagenase died of massive edema and brain herniation by 24 hours, while most of those injected with 0.5 unit survived. The greatest increase in water content was seen at the site of the hemorrhage, probably due to the large hematoma and the surrounding swollen tissue. At 4 and 24 hours, both posterior brain sections had edema, which had resolved by 48 hours. Behavioral testing showed abnormal total injury scores at 4, 24, and 48 hours, with improvement beginning at 48 hours.

The pathologic changes that were observed in the collagenase lesion are most consistent with those described for an intracerebral hemorrhage. The earliest change seen was an accumulation of erythrocytes around blood vessels. The blood cells were subsequently seen to dissect away from the blood vessels into intact brain tissue. Some necrotic vessels were seen in the lesion, but the erythrocytes appeared to move between brain cells. Collagenase-induced intracranial bleeding may be important in clinical conditions with leukocyte infiltration, tissue necrosis with release of intracellular proteolytic enzymes, and rapidly growing infiltrative malignancies. Enzymatic disruption of the extracellular matrix may occur in hemorrhagic infarctions, traumatic hemorrhages (early and late), and in brain tumors that bleed, such as metastatic melanomas.

Collagenases are a group of metalloproteinases that degrade interstitial and basement membrane collagen. The enzymes are released by macrophages and other mononuclear cells in response to injury and are able to act on the extracellular matrix at neutral pH. Immunocytochemical studies indicate that brain collagenase surrounds blood vessels.

Type IV collagen is found in the basal lamina surrounding brain blood vessels and in the pial lining of the Virchow-Robin spaces.

Collagenase-induced bleeding is a reproducible model that has features seen in intracerebral hemorrhage in humans, but on a compressed time scale. It should provide information on the response of brain tissue to bleeding and on the role of the basal lamina in the blood–brain barrier.

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References


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