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Research report

Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures

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Abstract

Reperfusion damages the bloodâ€“brain barrier (BBB). Matrix metalloproteinases (MMPs) are associated with the opening of the BBB, but their cellular localization and activation mechanisms are uncertain. We used immunohistochemistry to determine the cellular localization of the MMPs in reperfused rat brain, and cell cultures to study their activation. Spontaneously hypertensive rats (SHR) had a 90 min middle cerebral artery occlusion (MCAO) followed by reperfusion for times from 3 h to 21 days. Frozen sections were immunostained with antibodies to gelatinase A (MMP-2), stromelysin-1 (MMP-3), and gelatinase B (MMP-9). Sham-operated control rats showed MMP-2

(MMP-3), and gelatinase B (MMP-9). Sham-operated control rats showed MMP-2 immunostaining in astrocytic processes next to blood vessels. After 3 h of the onset of reperfusion MMP-2 immunostaining increased in astrocytes. At 24 h immunoreactivity for MMP-3 and MMP-9 appeared. MMP-3 co-localized with activated microglia (Ox-42+) and ischemic neurons (NeuN+). MMP-9 immunostaining was seen at 48 h in endothelial cells, neutrophils, and neurons. At 5 and 21 days intense MMP-2 staining was seen in reactive astrocytes around the ischemic core. Studies of activation of the MMP were done in lipopolysaccharide (LPS)-stimulated astrocyte and microglia cultures. Stimulated astrocytes produced an activated form of MMP-2. When microglia were stimulated, they activated MMP-9. Immunostaining showed MMP-3 in cultures of enriched microglial cells. The hydroxymate-type, MMP inhibitor, BB-1101, blocked the activation of MMP-2 and MMP-9 by LPS in mixed glial cultures. We propose that MMP-2 is normally present in astrocytic end feet, and that during ischemia MMP-9 and MMP-3 are produced. MMP-3 in microglia/macrophages may be activating proMMP-9. Our results show that a differential expression of MMPs by astrocytes, microglia, and endothelial cells at the blood vessels is involved in the proteolytic disruption of the BBB.

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Keywords

Astrocyte; Bloodâ€“brain barrier; Cerebral ischemia; Matrix metalloproteinase; Microglial cell; Stromelysin

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