MATRIX METALLOPROTEINASES (MMPS) ARE IMPLICATED IN THE EARLY ELEVATION OF INTERLEUKIN-1β (IL-1β) FOLLOWING TRANSIENT FOCAL BRAIN ISCHEMIA INDUCED BY MIDDLE CEREBRAL ARTERY OCCLUSION (MCAo) IN RAT

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IL-1β is an important mediator of neurodegeneration induced by cerebral ischemia (1). MMPs cleave protein components of the extracellular matrix, but also process a number of cell surface and soluble proteins including receptors, cytokines and chemokines (2). Here we investigate the putative involvement of IL-1β processing in the detrimental effects exerted by the early upregulation of MMPs in ischemic stroke. Brain ischemia was induced in male Wistar rats (280-320 g) by transient (2 h) MCAo. GM6001, a broad-range MMPs inhibitor, and its negative control (GMneg) were administered through the external carotid artery, 15 min prior to MCAo. Cerebral infarct volume was evaluated 24 h after MCAo by staining coronal brain slices with 2,3,5-triphenyltetrazolium chloride. Two hours after reperfusion, cortical tissue was dissected to measure pro-IL-1β immunoreactivity by western blotting and mature IL-1β levels by a rat specific sandwich ELISA (3). IL-1β cellular distribution was assessed by confocal microscopy on paraformaldehyde-fixed brain tissue using a polyclonal goat anti-rat IL-1β antibody (1:200). Gelatin and *in situ* zymography were performed, respectively, on cortical homogenates and fresh cryostat-cut sections of rat brains harvested after 2 h reperfusion (4).
In situ and gelatin zymography revealed a significant increase in MMP-2 and -9 activity in the ischemic cortex and striatum after 2 h MCAo followed by 2 h reperfusion. Increased gelatinase activity in the ischemic cortex was coincident with elevation of mature IL-1β (contralateral cortex: 5.6±1.0, ipsilateral cortex: 8.4±0.2 pg mg protein^{-1}, $P<0.05$). At this early stage of injury, IL-1β immunoreactivity increased mainly in astrocytes and in a few activated microglial cells in the ischemic side of the brain. Canonical caspase-1-dependent processing of pro(31 KDa)-IL-1β did not appear to be required to yield mature (17 KDa) IL-1β. Quite importantly, GM6001 (0.5 μg, i.a.), but not its negative control, abolished the early IL-1β increase in the ischemic cortex, reduced the cleavage of the cytokine pro-form and resulted in a significant reduction of ischemic brain volume (GMneg: 503±18, GM6001: 252±9 mm^3, $P<0.001$) supporting the deduction that MMPs are involved in the pathophysiology of neuroinflammation and damage to the brain undergone transient focal ischemia.

1. Rothwell N. (2003) Brain Behav. Immunity 17:152-157.
2. Sternlicht M.D. and Werb Z. (2001) Annu. Rev. Cell Dev. Biol. 17:463-516.
3. Corasaniti M.T., Turano P., Bilotta A. et al. (2001) J. Neurochem. 78:611-8.
4. Gu Z., Kaul M., Yan B. et al. (2002) Science 297 :1186-90.