Matrix Metalloproteinase-9 and Recovery of Acute Ischemic Stroke

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Background: Stroke outcome can be predicted by clinical features, biochemical parameters, and some risk factors. Matrix metalloproteinase-9 (MMP-9) is involved in various stages of stroke pathology. MMP-9 inhibitors are potential stroke therapeutic agents. Little is known about the relation between MMP-9—after the acute stage—and clinical recovery. *Objective:* The study aimed to investigate the serum level of MMP-9 at stroke onset as predictor of stroke outcome and the relation between the level of MMP-9 after 30 days and stroke recovery. *Methods:* The National Institutes of Health Stroke Scale, modified Rankin Scale, and serum level of MMP-9 were assessed in 30 patients with acute ischemic stroke during the first 24 hours of onset and then a month later. None of the patients received thrombolytic therapy. Thirty normal volunteers of matched age and sex were included in the control group. *Results:* The serum level of MMP-9 at stroke onset was independently positively correlated with stroke outcome. The serum level of MMP-9 30 days after stroke onset was positively correlated with initial stroke severity and outcome, as well as with clinical recovery. *Conclusion:* Higher serum level of MMP-9 at stroke onset can be a predictor of poor stroke outcome. However, beyond the acute stage, MMP-9 may play beneficial role in stroke recovery. **Key Words:** Matrix metalloproteinase—stroke—outcome—predictor—recovery—NIHSS—modified Rankin Scale.

Introduction

Neuronal cell death during ischemic stroke triggers an immune response leading to inflammatory cell activation and infiltration. These activated inflammatory cells release cytotoxic agents, including matrix metalloproteinases (MMPs), which may induce further cell damage and disruption of the blood–brain barrier. Proenzyme activation and the enzyme activities of MMPs are tightly regulated by tissue inhibitors of MMPs. MMPs are also expressed during development and contribute to morphogenesis of the central nervous system (CNS).[1] They can be critical for CNS recovery because they can remodel components of the extracellular matrix. MMPs participate in dendritic and axonal extension, as well as blood vessel formation.[2] Matrix metalloproteinase-9 (MMP-9) is a member of the MMP family that normally remodels the extracellular matrix. Its expression increases significantly after cerebral ischemia and is related to blood–brain barrier disruption, edema formation, or hemorrhagic transformation.[3-5] MMP-9 gene is one of the highly expressed genes in leukocytes after stroke.[6,7] MMP-9 is directly involved in the excitotoxic neuronal loss.[8]

Tissue inhibitors of metalloproteinases-1 (TIMP-1), which inhibit MMP-9, have neuroprotective effects after excessive glutamate stimulation.[9] Natural MMP inhibitors, treatment with MMP monoclonal antibodies,[10] genetic approaches[11] and other MMP-9 inhibitors may reduce ischemic damage.[12] The protective effects of hypothermia and

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hyperbaric oxygen, statins, minocycline, doxycycline, indomethacin, angiotensin-II receptor blocker (olmesartan), and estrogens on the blood–brain barrier, after ischemic brain injury, are likely to be also related to the decrease and inhibition of MMP-9.[4,12-21] The neuroprotective activity of calpain or cathepsin B inhibitor and antioxidants like quercetin against cerebral ischemia could also be attributed to their properties against MMP-9,[22-24] and the anti-hemorrhagic effect of PJ34, a potent poly(ADP-ribose) polymerase inhibitor, was associated with a 57% decrease in MMP-9 overexpression.[25]

Tissue plasminogen activator (tPA) significantly increases the expression of MMP-9 after ischemia.[26] It is possible that interactions between the tPA and MMP systems underlie some of the complications associated with tPA stroke therapy.[27] MMP-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis.[28] Several potential drugs for reducing tPA-related hemorrhagic complications (e.g., minocycline) have been identified. Many involve inhibition of MMP-9 activity.[29,30]

The present study aimed to estimate the serum MMP-9 level during the first 24 hours following ischemic stroke and after 1 month of follow-up, and to investigate its possible relation to clinical improvement and functional outcome after acute ischemic stroke.

Subjects and Methods

Subjects

This study was conducted on 30 consecutive patients with acute ischemic stroke who were recruited—within 24 hours from stroke onset—from the neurology inpatient ward, neurology outpatient clinic, and internal medicine department of Kasr El-Aini Teaching Hospital. Thirty normal volunteers of matched age and sex were the control subjects. All subjects provided informed consent. The study was approved by the local ethics committee, in accordance with the Declaration of Helsinki. This is a follow-up study; these subjects were previously studied to address the relation between MMP-9 and stroke criteria in acute setting (first 48 hours only). The age of patients ranged from 48 to 73 years, with a mean of 61 ± 7.11 years, whereas the age of the control subjects ranged from 53 to 79 years, with a mean of 63 ± 6.78 years. Patients were selected according to the following inclusion criteria: age ≥ 45 years and if patients presented within 24 hours from stroke onset (stroke was defined, according to the World Health Organization definition, as rapidly developing clinical signs of focal [or global] disturbance of cerebral function, lasting more than 24 hours or leading to death with no apparent cause other than of vascular origin).[31] The time of onset of stroke was defined as the time when the patient or observer first became aware of the symptoms, or the last time of symptom freedom in case of stroke onset during sleep. The following were the exclusion criteria: patients with hemorrhagic stroke; patients with atrial fibrillation and patients with other suspected cardiac origin of an embolic stroke; patients with severe systemic illness, for example, chronic renal failure, hepatic failure, malignancy, congestive heart failure, myocardial infarction, and other medical conditions that can increase the level of MMP-9[32,33]; and patients with history of regular intake of drugs that may affect the MMP-9 level, for example, tetracycline derivatives,[16,34] nonsteroidal anti-inflammatory drugs,[35] or statins.[36] As MMP-9 is also involved in arterial remodeling in arterial hypertension and atherosclerosis,[37] we included cases where atherosclerosis of cerebral vasculature is the most probable stroke etiology. Cardiological assessment including electrocardiography and transthoracic echocardiography were done to help in exclusion of cardioembolic causes of stroke.

Methods

Clinical and Routine Laboratory Assessment at Stroke Onset

Patients were subjected to thorough medical and neurologic examination. Stroke severity and dependency were evaluated with the National Institutes of Health Stroke Scale (NIHSS)[38] and the modified Rankin Scale (mRS).[39] The assessment was done within 24 hours of the event. Initial stroke severity was graded using the NIHSS score as mild (0-5), moderate (6-13), or severe (≥14).[40] Laboratory investigation of both cases and control subjects included fasting blood sugar, kidney and liver function tests, and other laboratory work to rule out other severe medical illness, serum uric acid, and total serum cholesterol. All patients were treated by the same team according to the American Heart Association–American Stroke Association Guidelines for the Early Management of Patients with Acute Ischemic Stroke.[41]

Imaging Studies

Brain computed tomography was done on all patients in the radiology department of New Kasr El-Aini Teaching Hospital using the General Electric (GE) Light Speed VCT (GE Healthcare. 4380 Brockton Dr SE, Kentwood, MI 49512, USA). Axial computed tomography cuts were performed while patients were lying in the supine position, with 15°-20° tilt with 1 cm slice thickness, in addition to posterior fossa cuts every .5 cm. The size of infarction was determined by the largest diameter of the lesion according to Pan et al.[42] Magnetic resonance imaging was performed when further needed. All patients were subjected to B-mode and color-coded duplex sonography of the carotid[43] and vertebral arteries using the Phillips HDI 5000 (Phillips, 45-47 Brook St, London, W1K 4HN, England) ultrasound equipment. Extracranial vessels were evaluated by real-time imaging using a linear 10 MHz transducer. Examination with B-mode transverse scanning of the vessels was done to examine the arterial wall