Elevated Serum High-Mobility Group Box-1 Protein Level Is Associated with Poor Functional Outcome in Ischemic Stroke

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Background: In experimental models, inhibition of high-mobility group box-1 (HMGB1) signaling has been reported to protect against the sequelae of ischemic stroke. Here, we determined the clinical significance of serum HMGB1 levels in patients with acute ischemic stroke. *Methods:* We enrolled 183 patients (114 men, 69 women; mean age: 72.7 years) over 6 consecutive months. On admission and day 7, we recorded the National Institutes of Health Stroke Scale scores and measured serum high-sensitivity C-reactive protein (hs-CRP) and HMGB1 levels. Stroke volumes were estimated using diffusion-weighted magnetic resonance imaging performed on admission. One year later, clinical outcome was assessed using the modified Rankin Scale (mRS). *Results:* Serum hs-CRP and HMGB1 levels in patients with ischemic stroke were increased relative to healthy controls (both $P < .01$). On day 7, hs-CRP, but not HMBG1, levels had increased significantly relative to levels at admission ($P < .01$ and .54, respectively). Higher HMGB1, but not hs-CRP, levels at day 7 correlated with larger stroke volumes ($P < .01$ and .28, respectively). HMGB1 levels did not significantly differ between stroke subtypes. Multiple logistic regression analysis indicated that a serum HMGB1 level higher than 7.5 ng/mL was an independent risk factor for poor prognosis, defined as a 1-year mRS score of 3-6 (odds ratio, 2.34; 95% confidence interval, 1.02-5.38). *Conclusions:* Acute ischemic stroke is associated with elevated serum HMGB1 levels, and HMGB1 levels at admission independently predict poor outcome at 1 year. These results suggest that HMGB1 quantification provides more accurate prognostic information after ischemic stroke. **Key Words:** Prognosis—cerebral ischemia—HMGB proteins—inflammation—cerebral infarction.

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Introduction

In ischemic stroke, cerebral artery occlusion leads to nutrient and oxygen depletion, as well as necrosis and subsequent inflammation in neural tissue.[1] This postischemic inflammation accounts for a significant portion of pathogenic stroke progression.[1,2] Cellular cytotoxicity resulting primarily from inflammation can induce cell death and permanent tissue damage in the parenchyma surrounding the necrotic core tissue, defined as the penumbra of the lesion.[2,3]

Although recanalization of blocked arteries by intravenous thrombolytic administration (e.g., recombinant tissue plasminogen activator) is a well-established treatment for ischemic stroke, thrombolytic therapies require immediate application within several hours of onset.[4,5] Additionally, recombinant tissue plasminogen activator has adverse effects, including life-threatening bleeding into the ischemic tissue.[6,7]

The inflammatory response rapidly induced by ischemic cerebral tissue stimulates brain cells and immune cells by recruiting multiple cytokines and chemokines and upregulating adhesion molecule expression.[8] In particular, injured brain cells release danger-associated molecular patterns (DAMPs) that induce or escalate inflammation.[9] These DAMPs include high-mobility group box-1 (HMGB1) among others.

The postischemic stroke inflammatory response consists of 2 phases: the early phase, which consists of neural tissue destruction, and the late phase, which consists of tissue remodeling.[10] Despite this complexity, increasing evidence suggests that inhibition of inflammation can be a therapeutic target for further reducing neurological deficits after ischemic stroke.[11] Due to the clinical limitations of thrombolytic therapies mentioned earlier, medical interventions targeting poststroke inflammation have been developed and have shown promise as an alternative treatment for ischemic stroke.[1,3] For example, blockade of either interleukin (IL)-1β or tumor necrosis factor-alpha reduces infarct size and ameliorates neurological deficits relative to controls in an animal model.[12,13]

HMGB1 is a ubiquitous nuclear protein with proinflammatory activity in sepsis and ischemia.[14,15] Under ischemic conditions, HMGB1 is passively released from necrotic cells and actively secreted by stimulated inflammatory cells.[16] Once released, HMGB1 acquires proinflammatory activity and acts as a DAMP.[9,10] In an experimental animal model, extracellularly secreted HMGB1 provoked an inflammatory response via blood–brain barrier disruption and increased vascular permeability.[17] Recently, inhibition of HMGB1 activity has been reported to have a protective effect against damage following ischemic stroke, in that the administration of anti-HMGB1 neutralizing antibodies reduced infarct volume and ameliorated infarction after experimental middle cerebral artery occlusion in rats.[18] Blockade of HMGB1 using siRNA also reduced infarct volume.[19] These studies demonstrate that HMGB1 is a key immune mediator of cerebral stroke, at least in these models.[20]

To date, many studies have shown that inflammatory responses are involved in the pathophysiology of myocardial infarction.[21,22] In patients with acute coronary syndromes, serum HMGB1 concentration is associated with increased mortality and cardiac infarct size.[23,24] However, there are few studies showing the clinical associations between HMGB1 and ischemic stroke, although serum or plasma HMGB1 concentration is known to be elevated in acute stroke, as well as in acute coronary syndrome.[8,25,26] Therefore, the present study sought to elucidate clinical factors associated with HMGB1 in patients with acute ischemic stroke.

Methods

Patient Selection

We prospectively included consecutive patients (n = 183) with acute ischemic stroke admitted via the emergency department of the Japanese Red Cross Nagoya Daini Hospital from September 2011 to March 2012. Ischemic stroke was defined as an acute focal neurological deficit and confirmed with initial diffusion-weighted magnetic resonance imaging (DW-MRI). Patients were included if they were admitted with confirmed stroke within 24 hours of symptom onset. Exclusion criteria were as follows: (1) patients aged under 18 years; (2) patients who underwent surgery after admission; and (3) patients with brain tumors, intracerebral hemorrhage (chronic subdural hematoma), or bone fractures (femoral fracture and lumbar compression fracture). There were 188 patients with acute ischemic stroke who met the inclusion criteria in our study. Among these 188 patients, 5 patients declined to provide informed consent. Thus, 97.3% of the screened patients consented to participate in the present study. The study was approved by the ethics committee of the Institutional Review Board of Nagoya Daini Red Cross Hospital, and all patients provided written informed consent. The inclusion criteria for healthy controls were as follows: (1) age over 18 years, (2) no disability restricting activities of daily living, and (3) no past medical history. After having given informed consent, volunteers were recruited to serve as healthy controls among the staff of the Japanese Red Cross Nagoya Daini Hospital. We included 16 healthy controls (8 men and 8 women) in the present study. The mean age of the healthy controls was 33.6 ± 9.6 years (range, 25-56 years).

Data Collection and Patient Follow-Up

All patients were examined by neurologists and admitted to the stroke unit. At admission, we recorded demographic and clinical features, including risk factors, medication history, vital status (body temperature, systolic