Effects of Edaravone, a Free Radical Scavenger, on Circulating Levels of MMP-9 and Hemorrhagic Transformation in Patients with Intravenous Thrombolysis Using Low-dose Alteplase

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Background: Matrix metalloproteinase-9 (MMP-9) plays a key role for the blood–brain barrier disruption and intravenous tissue plasminogen activator (iv-tPA) therapy increases MMP-9. Edaravone, a free radical scavenger, reduces MMP-9–related blood–brain barrier disruption. We aimed to investigate whether edaravone would suppress the MMP-9 increase after iv-tPA using low-dose alteplase (0.6 mg/kg). *Subjects:* Patients hospitalized within 12 hours after ischemic stroke onset between April 2008 and June 2013 were retrospectively examined. Patients with slight deficits (National Institutes of Health Stroke Scale score ≤4), stroke caused by arterial dissection, severe inflammatory disease or autoimmune disease, or regular use of steroid were excluded. Serum concentrations of high-sensitivity C-reactive protein, interleukin-6, MMP-2, and MMP-9 were serially measured at admission, after 24 hours, day 7, and day 14. General linear models were used to compare changes in concentrations of these biomarkers over time. *Results:* A total of 63 patients (38 men, aged 74.48 ± 13.8 years) were studied. Patients were divided into 2 groups according to the iv-tPA therapy, that is, tPA group (n = 32) and non-tPA group (n = 31). Edaravone was administered routinely except for contraindication (90.6% in the tPA group and 87.1% in the non-tPA group). Significant interaction of group × time factor was observed only in MMP-9 concentrations by repeated-measure analysis of variance ($P = .004$). Association between iv-tPA therapy and subsequent hemorrhagic transformation was highly significant, but MMP-9 concentrations at any point did not predictive of subsequent hemorrhagic transformation (area under the receiver operating characteristic curve, .681). *Conclusions:* Low-dose iv-tPA increases MMP-9 concentration even in combination with Edaravone. The effect of higher dosage of Edaravone on circulating MMP-9 concentration and subsequent hemorrhagic transformation should be investigated. **Key Words:** Tissue plasminogen activator—matrix metalloproteinase-9—hemorrhagic transformation.

Edaravone, a free radical scavenger, eliminates free radicals produced during ischemic reperfusion in various experimental models[1-4] and has inhibitory effects on matrix metalloproteinase-9 (MMP-9) expression in the ischemic brain.[5,6] Since its approval as a cerebroprotective agent in 2001, Edaravone has been widely used in Japan to treat ischemic stroke within 24 hours of onset and appears to show effects on inflammatory reactions in combination with free radical scavenging.[7-9] Several lines of evidence have shown that intravenous administration of tissue plasminogen activator (iv-tPA) increases both

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brain levels and circulating levels of MMP-9, which mediates leakage of the blood–brain barrier and facilitates brain edema and hemorrhage.[10-12] These studies suggest that combination therapies of an MMP-9 inhibitor[13-17] or indirect inhibition by free radical scavengers with iv-tPA therapy may be effective.[18] We investigated whether iv-tPA using low-dose alteplase (.6 mg/kg) in combination with Edaravone administration suppresses an increase in the MMP-9 concentration and if MMP-9 levels are predictive of subsequent hemorrhagic transformation.

Patients and Methods

We retrospectively examined 63 patients (38 men and 25 women; mean age of 74.5 ± 13.8 years) who were hospitalized within 12 hours of the onset of ischemic stroke between April 2008 and June 2013. Diffusion-weighted magnetic resonance imaging was performed in all patients. According to the Japanese stroke guidelines, except for patients with specific contraindications, all patients admitted within 24 hours received Edaravone infusion with a dosing regimen of 30 mg twice a day for a maximum duration of 14 days, and iv-tPA therapy with low-dose alteplase (.6 mg/kg) was administered within 3 hours after onset in patients without any specific contraindications. We excluded patients with: (1) slight neurologic deficits at admission (National Institutes of Health Stroke Scale [NIHSS] score <4), (2) serious concurrent disease, (3) stroke caused by arterial dissection or uncommon disease, (4) severe inflammatory disease or autoimmune disease (white blood cells $>1.0 \times 10^4$ mm^3, C-reactive protein >3.0 mg/dL), and (5) use of steroids or nonsteroidal anti-inflammatory drugs.

On admission, demographic data, including history, comorbidity, medical treatment before admission, and neurological deficit (NIHSS score) were recorded. Based on clinical data including neuroimaging data, the stroke subtype was diagnosed on admission according to the Trial of Org 10172 in Acute Stroke Treatment classification.[19] The subtype of hemorrhagic transformation was classified into four categories according to the European Cooperative Acute Stroke Study II criteria: (1) HI-1: small petechiae along the margins of the infarct; (2) HI-2: more confluent petechiae within the infarct area but without a space-occupying effect; (3) PH-1: blood clots in ≤30% of the infarcted area with some slight space-occupying effect; (4) and PH-2: blood clots in >30% of the infarcted area with substantial space-occupying effect.[20]

Standardized assessment of all patients was performed on admission, 24 hours, and 7 days after onset, and at discharge by the attending neurologist. Patients were divided into 2 groups according to the iv-tPA therapy, that is, tPA group (n = 32) and non-tPA group (n = 31). This study was conducted in a single hospital, and the study protocol was approved by the St. Marianna University Bioethics Committee.

Measurement of Biomarkers

Peripheral blood samples were obtained by venipuncture within 60 minutes of arrival at the hospital. All blood samples were centrifuged at 3000 rpm for 10 minutes at 4°C. Serum was separated and stored at −80°C until analysis. High-sensitivity CRP (hs-CRP), interleukin (IL)-6, MMP-2, and MMP-9 levels were measured twice at once with an enzyme-linked immunosorbent assay (ELISA) using commercially available ELISA kits, namely the CardioPhase™ High sensitivity C-Reactive Protein system from Siemens, the Biotrak™ high sensitivity human IL-6 ELISA system from GE Healthcare, the human MMP-2 Kit from Daiichi Fine Chemical, and the human MMP-9 ELISA System from GE Healthcare. Circulating levels of biomarkers including MMP-9 were serially measured at admission, after 24 hours and on day 7. Detectable ranges in healthy controls were determined with reference to data supplied by the manufacturers.

Statistical Analyses

Characteristics of patients are given as the mean and standard deviation, unless otherwise indicated. Unpaired Student's t tests were used to compare continuous variables, and χ^2 tests were used for nominal parameters. The Mann-Whitney test was used for data that were not normally distributed. Values of $P < .05$ were considered significant. Two-way repeated-measures analysis of variance (ANOVA) was used to compare changes over time in concentrations of biomarkers between groups. Lack of correlation was evaluated using the Mauchly sphericity test. If this assumption was not satisfied, the Greenhouse-Geisser correction was used at a significance level of $P < .05$.[21] The predictive value of MMP-9 levels was tested using receiver operating characteristic curve analysis. A 2-sided P value of <.05 was considered statistically significant. All statistical analyses were performed using SPSS version 21 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY).

Results

No statistically significant differences in patient characteristics were found between the 2 groups, except the time from onset to admission (Table 1). According to the Japanese guidelines, Edaravone was routinely administered to all patients except for those with a contraindication. Therefore, the frequency of Edaravone treatment in both groups was very high, that is, 90.6% in the tPA group and 87.1% in the non-tPA group. The frequency of patients with hemorrhagic transformation in the tPA group was significantly higher than that in the non-tPA group ($P = .004$, Table 2). Serial changes in biomarkers are shown in Table 3. A significant difference was observed in hs-CRP at 24 hours after onset and in baseline IL-6 between the 2 groups ($P = .018$ and .043, respectively).