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The free-radical scavenger edaravone accelerates thrombolysis with alteplase in an experimental thrombosis model

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ABSTRACT

Background and Purpose: Reperfusion injury after thrombolytic therapy can have adverse neurologic effects. The free-radical scavenger edaravone is used in combination with the recombinant tissue plasminogen activator alteplase to treat acute ischemic stroke. However, basic investigations of this combination use remain inadequate. Here, we used an in vivo model to investigate the effects of edaravone on alteplase-induced thrombolysis.
Methods: Thrombolysis was evaluated by using a He-Ne-laser–induced thrombosis model in rat mesenteric microvessels. Changes in thrombus volume were analyzed with the image analysis software Image-Pro Plus (Media Cybernetics, USA).
There were three experimental groups (placebo, alteplase 0.6 mg/kg, alteplase 0.6 mg/kg + edaravone 10.5 mg/kg). Sequential changes (0 to 60 min) in thrombus volume were compared by using a relative optical density method that we had used previously.
Results: In the placebo group, the thrombus volume at 60 min, reflecting the extent of thrombolysis, was 97.2% ± 5.7% of the initial value. In the alteplase group, thrombus volume decreased to 70.7% ± 4.1% ($P < 0.01$) after 20 min and 14.2% ± 6.6% after 60 min. In the alteplase + edaravone group, thrombus volume decreased to 66.9% ± 7.2% ($P < 0.001$) after 10 min and 10.9% ± 2.3% after 60 min.
Conclusions: These results support the hypothesis that edaravone accelerates thrombolysis by alteplase.

Introduction

Cerebral circulatory diseases are leading causes of morbidity and mortality in the developed world and in many developing countries [1,2]. The recombinant tissue-type plasminogen activator (rt-PA) alteplase is the only medication approved for the treatment of acute ischemic stroke (AIS). This therapy was approved by the Food and Drug Administration in 1996 for selected patients who can be treated within 3 h of stroke onset. The approval was based on the results of a clinical trial sponsored by the US National Institute of Neurological Disorders and Stroke [3,4]. In recent years, alteplase has been studied in several trials to assess its effectiveness in peripheral vascular thrombolysis [5–9]. Alteplase is increasingly becoming the thrombolytic agent of first choice for the treatment of peripheral arterial and venous thrombosis [3,10].

The only approved treatment for patients with AIS is the recanalization of occluded cerebral arteries through thrombolysis induced by alteplase within the first few hours after the onset of neurologic symptoms [3,11,12]. Intravenous administration of alteplase has been proven to improve the neurologic outcomes of AIS [3,8]. However, although many studies have shown the efficacy of thrombolytic therapy with alteplase, many problems have remained, including hemorrhagic complications, neurologic injury induced by reperfusion, and potential neurotoxicity [13,14]. Especially, the reperfusion injury is clinically important. Although thrombolytic treatment with alteplase is beneficial, reperfusion injury from reflow must be prevented, or widespread brain damage may occur. If recovery of circulation is achieved as early as possible, the functional outcome in the ischemic penumbra can be good, but prolongation of ischemia causes irreversible cell injury. The most important way to rescue cells in the ischemic zone is to start treatment as soon as possible within the therapeutic time window. It is considered the fundamental therapeutic strategy is combination therapy with a thrombolytic agent and neuroprotective agent.

Neurologic injury can have far-reaching adverse effects on quality of life. Although several free-radical scavengers have been assessed for their efficacy as neuroprotective agents in AIS, most of these compounds have failed to prove clinically useful for this purpose [15,16]. In Japan, the free-radical scavenger edaravone has been administered within 24 h of the onset of AIS in patients with various subtypes of stroke, including lacunae, large-artery atherosclerosis, and cardioembolic stroke [17,18]. Clinical trial data have shown that administration of

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edaravone within 72 h of the onset of AIS significantly improves neurologic outcomes over a 3-month follow-up period [19]. Edaravone is now being used widely to treat AIS in Japan [20].

We reported previously that edaravone prevents the formation of He-Ne-laser–induced thrombi [21]. In addition, we have shown that edaravone enhances endothelial nitric oxide synthase (eNOS) expression on the endothelium and improves NO release from endothelial cells, thus contributing to the homeostasis of microcirculation [22]. These experimental data suggest a possibility that edaravone may have potentially thrombolytic efficacy additional to that of rt-PAs.

Here, we investigated the effects of combination with edaravone on alteplase-induced thrombolysis by using a He-Ne-laser–induced thrombosis model in vivo.

Materials and Methods

Experimental Animals

Male Wistar–ST rats weighing 250 to 330 g were obtained from Japan SLC, Inc. (Hamamatsu, Japan). All animals were maintained in air-conditioned rooms (temperature: 22.5 ± 0.5 °C; humidity: 50% ± 5%) with a 12-h light–dark cycle. Animals had free access to food and drinking water. All procedures were conducted in compliance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

Edaravone (Free-radical Scavenger)

Edaravone (MCI-186; [3-methyl-1-1-phenyl-2-pyrazolin-5-one) was kindly donated by Mitsubishi Pharma Corporation (Tokyo, Japan). Edaravone was administered to rats by infusion into the femoral vein over 60 min (10.5 mg/kg/h) by using an infusion pump. This dosage was selected based on human that had demonstrated the effectiveness of the drug [19].

Alteplase (Recombinant Tissue Plasminogen Activator)

Alteplase (GRTPA for injection) was kindly donated by Mitsubishi Pharma Corporation (Tokyo, Japan). Alteplase (6,000,000 IU/vial) was dissolved in distilled water and stored at −80 °C until use. Alteplase was administered to rats by infusion into the femoral vein (10% bolus plus 90% perfusion over a 45-min additional period) in 1 ml saline solution. The dosage (0.12, 0.3, 0.6 mg/kg; n = 8 animals in each group) was used to investigate dose-dependence. The dosage was tested and we found 0.6 mg/kg most appropriate and decided to use this.

Experimental Thrombolysis Model

Animals were anesthetized with sodium pentobarbital (60 mg/kg i.m.) and the femoral blood vessels exposed by a median incision. A cannula (PE 50, Becton Dickinson, USA) was introduced into the femoral vein for administration of the study agents, and another was introduced into the femoral artery to monitor blood pressure respectively. A loop of intestine was extracted through the midline abdominal incision and spread over a perspex plate. An O-ring was placed around the tissue to stop vessel movement during peristalsis. The perspex plate was attached to the stage of a microscope (Olympus BX51, Japan) and the preparation observed through a long-working-distance objective. Arterioles (30 to 35 μm in diameter) were selected for irradiation. The He-Ne laser beam was introduced into the microscope by using a dichroic mirror and focused on the center of each selected vessels. The diameter of the laser spot on the focal plane was 15 μm, and the power was 25 mW. Evans blue (1.6 mg/kg, E. Merck, Germany) was injected through the femoral vein cannula. Irradiation for 5 s was repeated every 30 s until a mural thrombus occluding 80% of the vessel lumen in monitor was formed. After stabilisation for 10 min, infusion of Edaravone (10.5 mg/kg) or saline began through the femoral vein cannula. Subsequently, the infusion of alteplase (0.12, 0.3, 0.6 mg/kg) (10% bolus plus 90% perfusion over a 45-min additional period) in 1 ml saline solution or saline into the femoral vein began.

Analysis of Thrombus Size

Thrombolysis was assessed by using a slight modification of our previously reported method [23,24]. Briefly, changes in thrombus volume were analyzed with the image analysis software Image-Pro Plus (Media Cybernetics, Rockville, MD). Two-dimensional outline images of thrombi were captured in situ on a computer at 5-min intervals. Subsequently, 3D images were constructed by establishing optical density values relative to that of an area of the blood vessel lumen not involved in thrombus formation (Fig. 1). Integrative optical density (IOD) values were computed corresponding to the thrombus size. Changes in thrombus size were calculated according to the following formula. Thrombus size = IODn ÷ IODo (IODn = the integrative optical density at various times intervals during thrombolysis; IODo = the integrative optical density immediately after the stabilizing thrombus). The extent of thrombolysis was expressed as a percentage of the initial thrombus volume.

Our experiments were performed in a masked manner. The investigators performing the surgery gave each group of animals (n = 8) a secret code that remained unknown to the experimenters in charge of

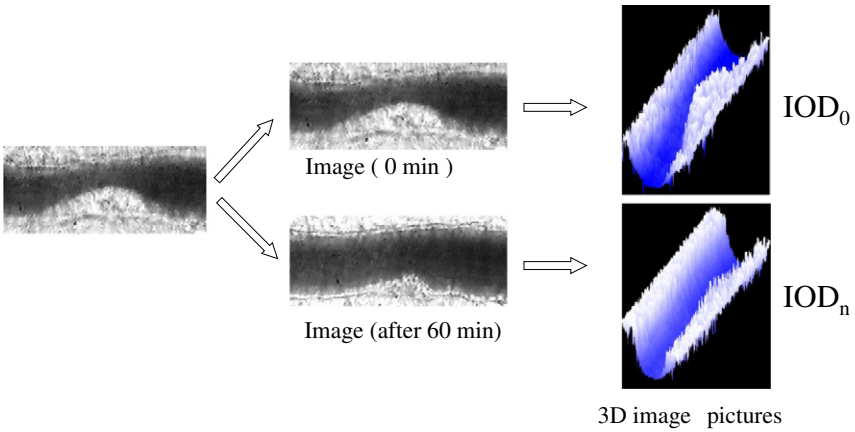

Fig. 1. Evaluation of thrombus size by using imaging software. Thrombus size = $IOD_n \div IOD_0$; IODn = the IOD at various time intervals during thrombolysis and IOD_0 = the IOD immediately after stabilization of the thrombus . The extent of thrombolysis was expressed as thrombus volume as a percentage of the initial thrombus volume.