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Therapeutic time window of tacrolimus (FK506) in a nonhuman primate stroke model: Comparison with tissue plasminogen activator

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Abstract

Tacrolimus (FK506), an immunosuppressive drug, has been shown to exert a potent neuroprotective activity when administered immediately after occlusion of the middle cerebral artery (MCA) in a nonhuman primate model of stroke. Here, we assessed the neuroprotective efficacy of tacrolimus with delayed treatment using the same model and compared with that of recombinant tissue plasminogen activator (rt-PA). Ischemic insult was induced by photochemically induced thrombotic occlusion of MCA in cynomolgus monkeys, and tacrolimus (0.2 mg/kg) and/or rt-PA (1.0 mg/kg) was intravenously administered 2 h after MCA occlusion. In another experiment, tacrolimus (0.1 mg/kg) was administered 4 h after MCA occlusion. Neurological deficits were monitored for 28 days after the ischemic insult and cerebral infarct volumes were measured with brain slices. With drug administration 2 h after the ischemic insult, tacrolimus significantly reduced neurological deficits and infarct volumes in the cerebral cortex without affecting the recanalization pattern in the MCA, however, rt-PA did not significantly improve neurological deficits or infarct volumes, even though it increased the recanalization rate of the occluded MCA. Combined treatment with tacrolimus and rt-PA exerted additional protection. Administration of tacrolimus 4 h after the ischemic insult still showed significant amelioration of neurological deficits. These results suggested that tacrolimus had a wider therapeutic time window than rt-PA in the nonhuman primate stroke model.

Keywords: Focal cerebral ischemia; Nonhuman primate; Therapeutic time window; Tissue plasminogen activator; FK506

Introduction

The FDA-approved thrombolytic therapy using rt-PA demonstrated improvement in recanalization of the occluded cerebral artery, when administered within first 3 h after onset of stroke (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). Furthermore, earlier treatment with rt-PA has been reported to be associated with increased benefit (Marler et al., 2000). However, one problem associated with the thrombolytic therapy using rt-PA is the limited time window for treatment and another is a concern about inducing intracerebral hemorrhage (ICH). While rt-PA has shown efficacy for neurological outcome when administered within 3 h, a decline in the efficacy have been observed when administered beyond 3 h after onset of stroke. In the European rt-PA trial in which the time window was extended to 6 h, the overall rate of symptomatic ICH was increased to 8.4% (Hacke et al., 1998). Thus, the therapeutic time window is the most critical issue in the treatment of acute ischemic stroke.

Neuroprotective strategies with drugs to maintain viability of ischemic neurons surrounding the core of infarcted tissue has been desired as additional therapies. In the neuroprotective therapy, it is reasonable to believe that the drugs working on the specific biochemical mechanisms have individual therapeutic time windows. Calcium overload and glutamate release occur very soon after stroke, and inflammatory response to brain injury or apoptotic cascade activate following the first event and maintain for several days (Barone and Feuerstein, 1999). Therefore, pharmacological approaches targeting the specific mechanisms possess its therapeutic opportunity (Barone and Feuerstein, 1999). A potent immunosuppressant tacrolimus

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(FK506) has been reported to have potent neuroprotective properties in rodents as well as primate stroke models (Bochelen et al., 1999; Furuichi et al., 2003a,b; Sharkey and Butcher, 1994; Yagita et al., 1996). Because tacrolimus possesses multiple modes of action for neuroprotection, such as inhibition of apoptotic and necrotic cell death, attenuation of leukocyte accumulation and attenuation of microglia activation (Herr et al., 1999; Furuichi et al., 2004, Tsujikawa et al., 1998; Wakita et al., 1998; Wang et al., 1999), it is expected that tacrolimus could have a long therapeutic time window in stroke patients whose severity and progress are heterogeneous. In our previous studies using rodent stroke models, tacrolimus showed a therapeutic time window of 1 to 2 h in reducing the infarct volume (Furuichi et al., 2003a). Because of failures in many clinical trials of neuroprotectants for stroke in the past, however, it is not prudent to design a clinical protocol of a new drug only based on the results from the rodent models in determining the time window.

We have previously demonstrated that tacrolimus ameliorated neurological deficits in a primate stroke model (Furuichi et al., 2003b), the possible ultimate pre-clinical platform which has been recommended by the Stroke Therapy Academic Industry Roundtable (STAIR, 1999), however, the time window for tacrolimus has not been elucidated using this stroke model. We therefore decided to extend our study with this stroke model in monkeys to evaluate the long-term neuroprotective efficacy of tacrolimus with delay in initiation of treatment after cerebral ischemia, and compare its time window with that of rt-PA as well as combined treatment using both drugs. In the first set of the study, tacrolimus administered 2 h after the onset of middle cerebral artery (MCA) occlusion was evaluated with or without rt-PA, and neurological deficits were monitored for up to 28 days. Then, the second set of the study was carried out to see whether tacrolimus still exerted a neuroprotective effect when administered 4 h after the onset of ischemia.

Materials and methods

Animals

Experiments were carried out using 3- to 5-year-old male cynomolgus monkeys weighing 3.1–5.5 kg purchased from Shin Nippon Biomedical Laboratories (Kagoshima, Japan). Animals were housed individually in cages 60 cm wide by 70 cm high by 70 cm deep in accordance with the NIH requirement, at 23±2°C in temperature and 55±5% in humidity, under 12-h light/dark cycle (light on at 7:00 a.m.) for at least 1 week before experimental procedures. These studies were approved by the Ethics Committee for Laboratory Animal Experiments at Fujisawa Pharmaceutical Co., Ltd. (currently Astellas Pharma Inc.).

Surgical procedure

Surgery was carried out with the procedure described in our previous study (Furuichi et al., 2003b). Cynomolgus monkeys were lightly anesthetized by intramuscular injection of ketamine hydrochloride (10 mg/kg), tracheostomized and artificially ventilated immediately after intramuscular injection of atropine (0.05 mg/kg) and pancuronium bromide (0.05 mg/kg). Anesthesia was maintained with 0.6 to 1% isoflurane in a N_2O:O_2 gas mixture (N_2O:O_2=7:3) and immobilization was accomplished with 0.05 mg/kg of pancuronium bromide given intramuscularly every 2 h for up to 6 h after ischemia. The right femoral vein and artery were cannulated with a polyethylene tube (PE50) for administration of rose bengal or test drugs, and for measurement of blood pressure, heart rate, and blood gas parameters, respectively. The right MCA was occluded by a transorbital approach. Under an operating microscope, the temporalis muscle was sectioned, and subtemporal craniotomy was performed without removing the zygomatic arch. A window approximately 10 mm in diameter was opened just anterior to the foramen of the mandibular nerve at the skull base. The main trunk of the right MCA was visible through the window underneath the dura mater. After opening the dura mater, an optic fiber for green light irradiation was placed on the proximal site of the main MCA trunk, and the probe for a pulsed Doppler flowmeter (Model PDV-20, HHP-20, Crystal Biotech America, Northborough, USA) for blood flow measurement of MCA was placed next to the optic fiber. Photo-illumination with green light (wavelength; 540 nm, intensity; 4,000,000 lux) was achieved by using a xenon lamp (L-4887, Hamamatsu Photonics, Hamamatsu, Japan) with a heat absorbing filter and a green filter. Irradiation was directed by the optic fiber mounted on the MCA trunk, and rose bengal (20 mg/kg) was infused intravenously for 6 min. Photo-illumination was carried out for 20 min. Rectal temperature was maintained at 37.5±0.5°C with a heating pad (TR-100, PS-100, Fine Science Tools, Canada) during surgery and MCA blood flow monitoring afterward. After wound closure, buprenorphine hydrochloride (4 μg/kg) and cefazolin sodium hydrate (25 mg/kg) were intramuscularly administered to avoid pain and infection. Thereafter, anesthesia was stopped. After ascertainment of spontaneous breathing, each animal was returned to the home cage. The analgesic and antibiotic were given daily for 1 week.

Measurement of MCA blood flow

Complete MCA photothrombotic occlusion was confirmed by the decrease of blood flow monitored for 6 h following MCA occlusion by a pulsed Doppler flowmeter placed on the proximal MCA trunk. The time from rose bengal injection to complete occlusion of the MCA and the time of recanalization were measured to calculate the mean recanalization time of the MCA before and after drug treatment.

Assessment of neurological deficits

Animals were subjected to a neurological examination at 1, 2, and every 7 days for 28 days after MCA occlusion. Neurological assessment was performed blindly. As described