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Research Report

Modifying neurorepair and neuroregenerative factors with tPA and edaravone after transient middle cerebral artery occlusion in rat brain

*Kentaro Deguchi, Kazunori Miyazaki, FengFeng Tian, Ning Liu, Wentao Liu, Hiromi Kawai, Yosiho Omote, Syoichiro Kono, Taijun Yunoki, Shoko Deguchi, Koji Abe**

Department of Neurology, Graduate School of Medicine and Dentistry, Okayama University, 2-5-1 Shikata-cho, Okayama, 700-8558, Japan

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ABSTRACT

Changes in expression of neurorepair and neuroregenerative factors were examined after transient cerebral ischemia in relation to the effects of tissue plasminogen activator (tPA) and the free radical scavenger edaravone. Physiological saline or edaravone was injected twice during 90 min of transient middle cerebral artery occlusion (tMCAO) in rats, followed by the same saline or tPA at reperfusion. Sizes of the infarct and protein factors relating to neurorepair and neuroregeneration were examined at 4 d after tMCAO. The protein factors examined were: a chondroitin sulfate proteoglycan neurocan, semaphorin type 3A (Sema3A), a myelin-associated glycoprotein receptor (Nogo receptor, Nogo-R), a synaptic regenerative factor (growth associated protein-43, GAP43), and a chemotropic factor netrin receptor (deleted in colorectal cancer, DCC). Two groups treated by edaravone only or edaravone plus tPA showed a reduction in infarct volume compared to the two groups treated by vehicle only or vehicle plus tPA. Immunohistochemistry and western blot analyses indicated that protein expression of neurocan, Sema3A, Nogo-R, GAP43, and DCC was decreased with tPA, but recovered with edaravone. Additive edaravone prevented the reductions of these five proteins induced by tPA. The present study demonstrates for the first time that exogenous tPA reduced protein factors involved in inhibiting and promoting axonal growth, but that edaravone ameliorated such damage in brain repair after acute ischemia.

* *Corresponding author*. Fax: +81 86 235 7368.
E-mail address: degu@cc.okayama-u.ac.jp (K. Abe).

Abbreviations: CBF, cerebral blood flow; DCC, deleted in colorectal cancer; ECL, enhanced chemiluminescent; FLN, full-length neurocan; GAP43, growth associated protein-43; GFAP, glial fibrillary acidic protein; HE, hematoxylin-eosin; MAP2, microtubule-associated protein 2; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; Nogo-R, Nogo receptor; NVU, neurovascular unit; PAI-1, plasminogen activator inhibitor-1; PB, phosphate buffer; PBS, phosphate buffered saline; PS, physiologic saline; PVDF, polyvinylidene difluoride; rCBF, regional CBF; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SC, sham control; Sema3A, semaphorin 3A; tMCAO, transient middle cerebral artery occlusion; tPA, tissue plasminogen activator; TSA, tyramide signal amplification

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1. Introduction

Ischemic brain damage can be effectively ameliorated if cerebral blood flow (CBF) is restored by thrombolytic agents such as tissue plasminogen activator (tPA) within a short time (The NINDS t-PA Stroke Study Group, 1997). Endogenous tPA is a major parenchymal serine protease in the brain, and regulates physiological tissue remodeling and plasticity (Gravanis and Tsirka, 2005). However, a high dose of exogenous tPA could also cause hemorrhagic transformation through disturbance of the neurovascular unit (NVU) (Yamashita et al., 2009) and direct neurotoxicity (Lukic-Panin et al., 2010), presenting a threat to the safety of the thrombolytic therapy patients.

The adult central nervous system regenerates poorly after injury such as ischemia, which is partly due to protein factors inhibiting axonal growth in the peri-ischemic region (Fawcett and Asher, 1999). Among such factors, a chondroitin sulfate proteoglycan neurocan, semaphorin type 3A (Sema3A), a myelin-associated glycoprotein receptor (Nogo receptor, Nogo-R), a synaptic regenerative factor (growth associated protein-43, GAP43), and a chemotropic factor netrin receptor (deleted in colorectal cancer, DCC), have been demonstrated to play important roles in neurorepair and neuroregeneration (Hoke and Silver, 1996).

However, the detailed expression of these factors has not been studied in relation to exogenous tPA administration or even the neuroprotective agent edaravone. We previously demonstrated that expression of neurocan peaked in the peri-ischemic region at 4 days after transient middle artery occlusion (tMCAO) of rats (Deguchi et al., 2005). In the present study, therefore, we therefore examined the relationship between the protein factors listed above in association with neurorepair and neuroregeneration, and the effects of tPA and edaravone on the expression of these proteins after tMCAO.

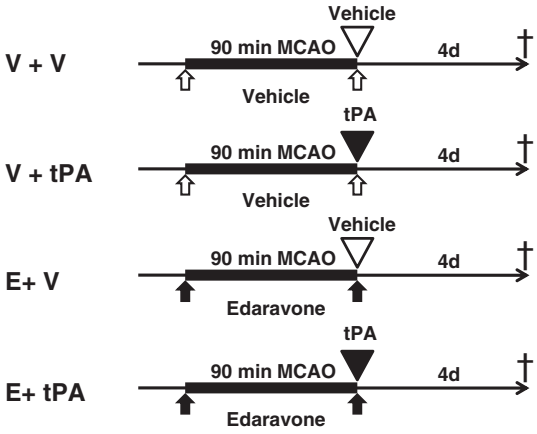

Fig. 1 – The four experimental groups are shown. The V (vehicle) + V (vehicle) group (n = 5) received saline twice during 90 min of MCAO (open arrows), then received the same amount of saline at reperfusion (open triangle). The V + tPA group (n = 5) received saline twice during 90 min of MCAO, then received tPA at reperfusion (filled triangle; 10 mg/kg). The E + V group (n = 5) received edaravone twice during 90 min of MCAO (filled arrows; 3 mg/kg) then received the same amount of saline at reperfusion. The E + tPA group (n = 5) received edaravone twice during 90 min of MCAO then received tPA at reperfusion. Rats were sacrificed 4 d after the reperfusion. E, edaravone; MCAO, middle cerebral artery occlusion; PS, physiologic saline; tPA, tissue plasminogen activator; V, vehicle.

2. Results

2.1. *Cerebral blood flow (CBF)*

Compared with regional CBF (rCBF) before middle cerebral artery occlusion (MCAO), rCBF immediately dropped to less than 30% of the basal level after MCAO. After reperfusion, rCBF quickly recovered to the basal levels in all experimental groups. Throughout the tMCAO experiments, there were no significant differences in rCBF between the 4 groups (data not shown).

2.2. *Cerebral infarct area*

The V (vehicle) + tPA group showed a small and not significant increase in the infarct size (263.3 ± 25.1 mm^3) compared to the V + V group (246.2 ± 7.8 mm^3). In contrast, the infarct volume (187.5 ± 13.4 mm^3) was significantly decreased in the E (edaravone) + V group compared with the V + V group ($^{**}p < 0.01$) and the V + tPA group ($^{\#\#}p < 0.01$). The E + tPA group also showed decreased infarct volume (221.8 ± 9.5 mm^3) compared with the V + tPA group ($^{\#}p < 0.05$), but not the V + V group. Examples and statistical data are shown in Figs. 2A–E.

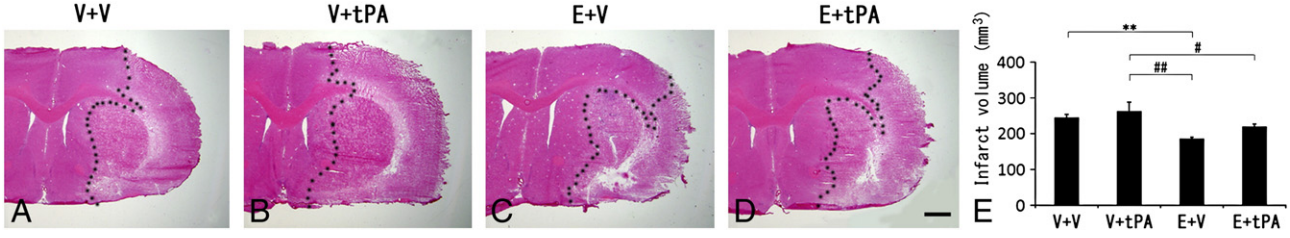

Fig. 2 – Representative HE staining of coronal sections with or without treatment (vehicle; V) of tPA and edaravone (E) at 4 d after tMCAO (panels A–D) and the infarct volumes (panel E). Note the reduction of infarct volume in the 2 edaravone groups. ($^{**}p < 0.01$, $^{\#}p < 0.05$, and $^{\#\#}p < 0.01$). Scale bar = 1 mm.