Thrombosis Research (2008) **122**, 247–255

ELSEVIER

THROMBOSIS RESEARCH

intl.elsevierhealth.com/journals/thre

REGULAR ARTICLE

Changes in tissue factor and the effects of tissue factor pathway inhibitor on transient focal cerebral ischemia in rats ☆

Masaki Niiro *, Tetsuya Nagayama, Shunji Yunoue, Soichi Obara, Hirofumi Hirano

Department of Neurosurgery, Graduate School of Medicine and Dental Sciences, University of Kagoshima, 8-35-1, Sakuragaoka, Kagoshima, 890-8520, Japan

Received 16 April 2007; received in revised form 1 October 2007; accepted 3 October 2007
Available online 11 December 2007

KEYWORDS
Tissue factor;
Focal cerebral ischemia;
Reperfusion injury;
Tissue factor pathway inhibitor;
Cerebral microcirculation

Abstract

Introduction: To determine the contribution of tissue factor (TF) to focal cerebral ischemia/reperfusion injury, we investigated the changes in TF in rat brains with transient focal cerebral ischemia and also assessed the effect of TF pathway inhibitor (TFPI).
Materials and methods: Spontaneous hypertensive rats were subjected to 90-min of middle cerebral artery occlusion (MCAO) and then were reperfused for up to 24 h. Immediately after MCAO, recombinant human TFPI (rhTFPI) (50 or 20 μg/kg/min) was administered by means of a continuous intravenous injection for 4.5 h.
Results and conclusions: TF immunoreactivity decreased or scattered in the ischemic area after reperfusion, however, an increased TF expression was observed in the microvasculature with the surrounding brain parenchyma and it peaked at 3 to 6 h, which coincided with the start of fibrin formation. On the other hand, total TF protein in ischemic area continued to exist and did not remarkably change until 24 h after reperfusion. At 24 h after reperfusion, the total infarct volume in the group treated with 50 μg/kg/min rhTFPI was significantly smaller than that in the controls (saline). Western blotting and immunohistochemical studies showed that rhTFPI treatment resulted in a decrease of fibrin in the ischemic brains and microvasculature. TF-mediated microvascular thrombosis is thus considered to

☆ Presentation Society for Neuroscience, 33rd Annual Meeting, New Orleans, 2003.

* Corresponding author. Tel.: +81 99 275 5375; fax: +81 99 265 4041.
E-mail address: niiromsk@m2.kufm.kagoshima-u.ac.jp (M. Niiro).

doi:10.1016/j.thromres.2007.10.021

contribute to focal cerebral ischemia/reperfusion injury. The continuous infusion of rhTFPI until a peak of TF-mediated microvascular thrombosis therefore attenuates the infarct volume by reducing fibrin deposition in the cerebral microcirculation.

Introduction

The microvascular responses to focal cerebral ischemia are rapid. Within 1–2 h of middle cerebral artery occlusion (MCAO), the primary microvessel permeability barrier is compromised [1–5]. Endothelial cells rapidly express leukocyte adhesion receptors. The appearance of several kinds of adhesion molecules and their counter-receptors in the endothelium of microvessels coincides with the initial movement of inflammatory cells into the ischemic region [2,6,7]. The local activation of platelets, leukocytes, and coagulation results in intravascular obstruction [6,7]. The accumulation of fibrin in the vascular lumen suggests that thrombin is generated intravascularly [2,6,8]. Tissue factor (TF), a potent endogenous procoagulant, is prominent in both cerebral tissue and perivascular cells [9], and it also catalyzes fibrin formation by generating thrombin from plasma coagulation factors. In addition, TF may contribute to the development of microvascular occlusion [8,10]. A disturbance in the microcirculation is associated with a reduced ischemic and/or post-ischemic cerebral blood flow and increased brain injury [8,10–12].

In the physiological state, immunohistochemical analyses have demonstrated TF antigen in the adventitia of the vessels but not in the endothelial cells [9,13,14]. However, in the pathological state, TF may be induced on endothelial cells by such stimuli as ischemia and inflammation [13–16]. TF is found in both cerebral tissue and on perivascular cells, and TF-mediated events may contribute to microvascular defects after rat focal cerebral ischemia [8–10]. However, the changes in the brain TF levels and the expression of TF in the microvasculature during cerebral ischemia remain to be elucidated.

Tissue factor pathway inhibitor (TFPI) inhibits the TF function by blocking the extrinsic coagulation pathway by forming an inactive VIIa/TF/factor Xa complex and thereby inhibiting thrombin and fibrin generation [14,17]. TFPI is an effective agent for limiting the postoperative paraplegia associated with spinal ischemia [18] and it strongly inhibits both hepatic and renal ischemia/reperfusion injury in rats [19,20]. The pretreatment of anti-tissue factor monoclonal antibody against focal cerebral ischemia/reperfusion has been reported to result in a significant reduction of intramicrovascular fibrin [8] and an increased reflow in microvessels [10], however, the changes in the infarct volume remain unclear.

We herein investigated the changes in TF in the brain and vasculature of rats subjected to focal ischemia and studied the effects of TFPI on focal ischemia/reperfusion injury.

Materials and methods

Animal preparation

All experimental procedures were conducted in accordance with the guidelines for the care and use of laboratory animals of the University of Kagoshima, Graduate School of Medicine and Dental Sciences, Kagoshima, Japan. Adult male spontaneously hypertensive rats (SHR) (Charles River, Japan) weighing from 200 to 230 g were anesthetized with 1–2% halothane in a nitrous oxide/oxygen mixture (50/50) and allowed to breathe on their own. The right femoral artery was catheterized to both monitor the arterial blood pressure and for blood sampling. Blood samples obtained after reperfusion were assayed by the Clinical

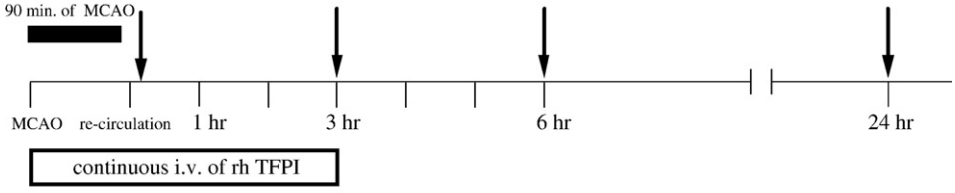

Figure 1 The flow diagram of the experiment.