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Research Report

Preischemic neuroprotective effect of minocycline and sodium ozagrel on transient cerebral ischemic rat model

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ABSTRACT

We investigated the neuroprotective properties of single doses of minocycline and ozagrel when administered prior to stroke. Male Sprague-Dawley rats were assigned randomly to one of the following groups: (1) control (Con) group ($n=10$), (2) minocycline (Mino) group ($n=10$), (3) sodium ozagrel (SO) group ($n=10$). Rats were treated with a single dose of minocycline or ozagrel at 30 min before stroke. A middle cerebral artery occlusion (MCAO) was made at 30 min after drug administration and reperfusion was done. The rats were subjected to a neurobehavioral test at days 1, 3 and 7 after MCAO. The cerebral ischemic volume was quantified by MetaMorph imaging software after TTC staining. The neuronal cell survival and astrocytes expansion were assessed by the NeuN and GFAP immunohistofluorescence staining. Apoptosis was detected by the TUNEL assay. We statistically analyzed and compared the results with each other. Mino and SO groups had neuroprotective effect and showed a better behavioral performance of adhesive removal and treadmill test at 7 days after stroke. Mino and SO groups also showed a smaller infarct volume than control group at 7 days after stroke. Immunohistofluorescence staining showed a higher number of NeuN positive cells, lower activated astrocytes in GFAP and a lower apoptosis in TUNEL staining. This study showed that single doses of minocycline and ozagrel prior to stroke had neuroprotective effects. These agents will be useful not only in post-stroke therapy but also in stroke prevention in several cerebrovascular procedures like carotid endarterectomy, bypass procedure, endovascular angioplasty, thromboembolectomy or thrombolysis.

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1. Introduction

Stroke is one of the most common causes of death worldwide and the most frequent cause of permanent disability. The poor prognosis of stroke is due to irreversible loss of brain cells before the patient receives medical attention, which is exacerbated by the void in delayed treatment options to protect against secondary injury. Cell death associated with the initial blood flow interruption and the immediately ensuing excitotoxicity are abrupt, while inflammation occurs over a long period of time from stroke onset. Accordingly, anti-inflammatory treatment is likely to extend the therapeutic window allowing improved intervention in stroke (Zhang et al., 2004).

Within several hours after stroke onset, ischemia induces inflammation that causes simultaneous alterations in the cerebrovasculatures including apoptosis and activation of microglia (Fawcett and Asher, 1999; Silver and Miller, 2004). Activated microglia and proliferated astrocytes release toxic substances such as nitric oxide (NO) and free radicals that can damage healthy neurons (Giulian, 1993) although some reports showed the beneficial role of microglia (Neumann et al., 2006, 2008). Free radicals generated during ischemia and reperfusion also play an important role in the development of brain damage via obstruction of cell membranes and formation of edema (Matsuo et al., 1995; Simonian and Coyle, 1996; Takamatsu et al., 1998). Platelet aggregation was also reported as one of the aggravating factors of cerebral ischemia (Suzuki et al., 1989). Thromboxane A_2 (TXA_2), a strong vasoconstrictor and platelet aggregator, is increased, and aggravates brain damage after cerebral ischemia–reperfusion (Chen et al., 1986; Petroni et al., 1989).

Minocycline is a semi-synthetic tetracycline with independent anti-inflammatory effects that is neuroprotective in models of brain injury including stroke (Domercq and Matute, 2004; Yrjanheikki et al., 1999). Neuroprotective effects of Minocycline include inhibition of gliosis, apoptosis, free radical formation, and peripheral inflammation (Fox et al., 2005; Tomas-Camardiel et al., 2004). Specifically, minocycline inhibits the post-ischemic induction of nitric oxide synthase (NOS) and reduces the release of cytochrome *c* by directly conferring stability to the mitochondrial membrane (Wang et al., 1998). Sodium ozagrel (ozagrel) is a selective TXA_2 synthase inhibitor. It ameliorates platelet aggregation, vasoconstriction and brain edema in acute cerebral ischemia (Chen et al., 1986).

Most of studies of minocycline and ozagrel are about the post-stroke therapeutic effect. However, many cerebrovascular procedures, like conventional cerebral angiography, carotid endarterectomy (CEA), balloon angioplasty or stenting, temporary block of cerebral circulation during cerebral aneurysm clipping or external carotid–internal carotid (EC–IC) bypass, often cause ischemic stroke (Bendszus et al., 1999; Britt et al., 2000; Enevoldsen et al., 1999; Kato et al., 2003; Soeda et al., 2003). Arterial vasospasm after subarachnoid hemorrhage can cause large cerebral infarct. The aim of this study is to assess the neuroprotective effect of minocycline and ozagrel when administered prior to these several neurovascular procedures which have a risk of ischemic stroke.

2. Results

2.1. Behavior evaluation

Fig. 1 shows behavioral results. Before ischemia, no rats showed a neurobehavioral deficit. Mino group or SO group showed more improved performance than control group in the adhesive removal test at 7 days after MCAO. In the adhesive removal test, there was a significant difference in the scores at 7 days in the Mino and SO group compared to the control group (Con: 129.90±17.00, Mino: 56.33±20.47, SO: 73.80±20.47 s, $p<0.05$) although there was no significant difference at day 1 (Con: 154.28±3.86, Mino: 159.00±15.32, SO: 152.40±15.20 s). Mino group or SO group also showed significant improvement in treadmill test at day 1 (Con: 29.47±6.95, Mino: 48.50±7.48,

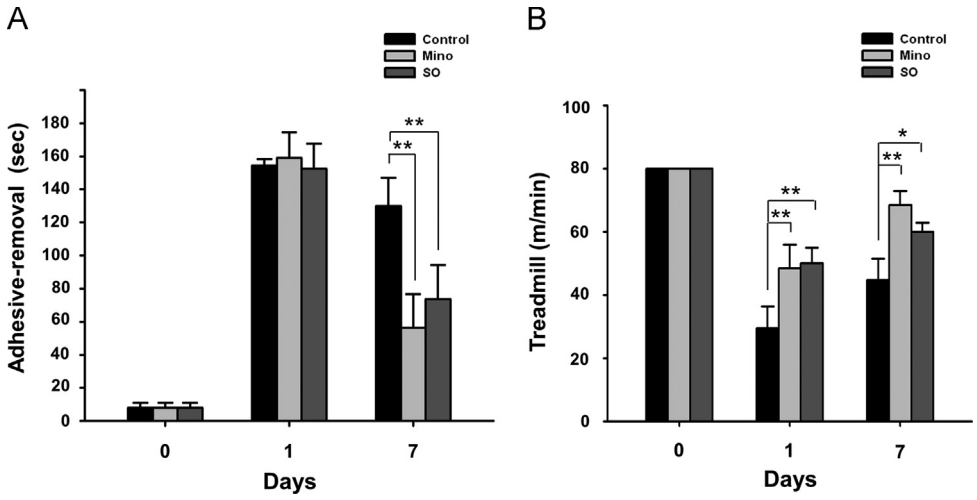

Fig. 1 – Behavior evaluations after middle cerebral artery occlusion (MCAO). Rats in Mino and SO group remove adhesive paper more quickly and more tolerable in treadmill test. The Mino group shows a slightly improved performance than the SO group although there is no statistical significance. *$p<0.05$, **$p<0.01$.