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Research Report

Cerebroprotective effects of TAK-937, a novel cannabinoid receptor agonist, in permanent and thrombotic focal cerebral ischemia in rats: Therapeutic time window, combination with t-PA and efficacy in aged rats

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ABSTRACT

Some occluded arteries of acute ischemic stroke (AIS) patients are not recanalized, even if thrombolytic therapy is performed. Considering such clinical settings, we examined the potential cerebroprotective efficacy of TAK-937, a novel cannabinoid receptor agonist, in young adult and aged rats with a permanent middle cerebral artery occlusion (MCAO) model and conducted a combination study with TAK-937 and tissue type plasminogen activator (t-PA) in a rat thrombotic MCAO model. TAK-937 significantly reduced infarct volume when it was administered 3 and 5 h after permanent MCAO in young adult rats. A thrombotic MCAO was induced by photo-irradiation of the middle cerebral artery with Rose Bengal administration and a permanent MCAO was produced by thermoelectric coagulation of occluded arteries. TAK-937 (10, 30 and 100 μg/kg/h) was intravenously infused 1, 3, 5, or 8–24 h after MCAO. t-PA (3 or 10 mg/kg) was intravenously administered 1, 1.5 or 2 h after MCAO. Infarct volume was determined using a 2,3,5-triphenyltetrazolium chloride staining method 24 or 48 h after MCAO. The combined treatment of TAK-937 with t-PA significantly reduced the cerebral infarction compared with t-PA treatment alone in a rat thrombotic MCAO model. TAK-937 reduced infarct volume of aged rats as well, when it was administered 1 h after permanent MCAO. These results suggest that TAK-937 exerts

Abbreviations: AIS, acute ischemic stroke; BT, body temperature; BDNF, brain derived neurotrophic factor; CB, cannabinoid; CNS, central nerve system; HPBCD, (2-Hydroxypropyl)-β-cyclodextrin; HR, heart rate; MABP, mean arterial blood pressure; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; $PaCO_2$, partial arterial carbon dioxide; PaO_2, partial arterial oxygen; sO_2, oxygen saturation; SD, Sprague–Dawley; t-PA, tissue-type plasminogen activator; TTC, 2,3,5-triphenyltetrazolium chloride

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protective effects regardless of age and has a wide therapeutic time window in permanent occlusion. Furthermore, combined treatment of TAK-937 with t-PA would provide more therapeutic efficacy compared to t-PA treatment alone.

1. Introduction

Stroke, resulting from the reduction of cerebral blood flow, is a major cause of death and is a leading cause of disability in the growing population of patients over the age of 60 years in the world. (Roger et al., 2012). Cerebral blood flow reduction is caused by an occlusion of the cerebral artery either by an embolus or a local thrombus that is most frequently generated in the middle cerebral artery (MCA). At present, tissue-type plasminogen activator (t-PA), a thrombolytic agent, is the only globally approved agent for treatment of acute ischemic stroke (AIS). When it is administered under the appropriate conditions, t-PA can significantly improve the symptoms of ischemic stroke patients by dissolving the embolus. However, due to its narrow therapeutic time window and the risk of promoting intracerebral hemorrhage, t-PA is administered to a very limited number of patients (The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995). Moreover, efficacy of t-PA is not sufficient since only a third of t-PA-treated patients recover and are free from disability. Therefore, novel therapeutic agents with better efficacy for acute ischemic stroke are eagerly required.

To date, two cannabinoid (CB) receptors have been identified: CB1, which is predominantly expressed in the central nervous system (CNS) (Herkenham et al., 1990; Matsuda et al., 1990), and CB2, which is predominantly expressed in peripheral tissues (Galiegue et al., 1995; Munro et al., 1993). Previously, it has been reported that cannabinoid receptor agonists showed pronounced protective effects against neuronal damage in vitro and in vivo (Nagayama et al., 1999). Intriguingly, the expression of CB1 receptors was significantly increased in the penumbra, the viable area surrounding the ischemic core, following transient MCAO (Jin et al., 2000). CB1 receptor agonists have been previously shown to inhibit intracellular calcium influx, reduce glutamate release and neuroinflammation, decrease stimulated iNOS expression, increase brain derived neurotrophic factor (BDNF), induce hypothermia, and exert antioxidant effects (Albayram et al., 2011; Grundy et al., 2001; Khaspekov et al., 2004; Mechoulam et al., 2002; Waksman et al., 1999; Zoppi et al., 2011). CB2 receptor agonists have been also reported to contribute to protection against ischemic brain damage by attenuating microvascular dysfunction (Murikinati et al., 2010; Zhang et al., 2009).

Under such background and the urgency for development of novel therapeutic agents for AIS, we identified TAK-937, a structurally novel, selective, and highly potent CB1/CB2 receptor agonist. We previously reported that TAK-937 showed dose-dependent cerebroprotective effects in a rat transient MCAO model. TAK-937 not only reduced infarct size, but also improved long term (4 weeks) neurological dysfunction and impairment of motor function in a rat transient MCAO model. Furthermore, TAK-937 showed protective effects after MCAO in cynomolgus monkeys (Suzuki et al., 2012a).

Considering the clinical settings mentioned above and to complement efficacy of TAK-937 in a model of transient MCAO, we conducted further studies to assess the potential of TAK-937 as a cerebroprotective agent. In this study, we examined the protective effect and therapeutic time window of TAK-937 in a rat model of permanent MCAO. We also examined the effect of TAK-937 combined with t-PA in a rat model of thrombotic MCAO. Moreover, we investigated whether the cerebroprotective effect of TAK-937 was replicated in permanent MCAO using aged-rats.

2. Result

2.1. *Physiological parameters*

To assess whether TAK-937 affects physiological parameters in young adult rats with a permanent MCAO, we monitored physiological parameters when TAK-937 (30 and 100 μg/kg/h) or vehicle was continuously administered from 1 h to 24 h post MCAO.

The physiological parameters in this study are summarized in Table 1. Before MCAO, no parameters were significantly different between TAK-937 and vehicle-treated groups. Similarly, just before administration of TAK-937 or vehicle after MCAO, no parameters were significantly different between TAK-937 and vehicle-treated groups. TAK-937 slightly, but not significantly, decreased mean arterial blood pressure (MABP), but significantly decreased heart rate (HR) and body temperature (BT) 6 h after MCAO compared to the vehicle-treated group. The changes of these parameters returned to normal level by 1 day after MCAO. Furthermore, administration of TAK-937 increased the partial arterial carbon dioxide ($PaCO_2$) and decreased the partial arterial oxygen (PaO_2) and oxygen saturation (sO_2) dose-dependently compared with the vehicle-treated group. On the other hand, TAK-937 did not alter the pH of arterial blood 6 h and 1 day after MCAO.

2.2. *Therapeutic time window and dose-dependent effect of TAK-937*

To explore the cerebroprotective effect and a therapeutic time window of TAK-937 in young adult rats with a permanent MCAO, administration of TAK-937 (30 and 100 μg/kg/h) was started from 3, 5 and 8 h after MCAO. The time course of this study is summarized in Fig. 1A. When it was administered 3 h after MCAO, TAK-937 significantly reduced the infarct volume to 81.2±3.4% (30 μg/kg/h) and 58.3±7.3% (100 μg/kg/h) TAK in