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Research Report

Trans-sodium crocetinate improves outcomes in rodent models of occlusive and hemorrhagic stroke

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ABSTRACT

Trans-sodium crocetinate (TSC) is a novel carotenoid compound capable of enhancing the diffusion of small molecules in aqueous solutions. TSC improves the diffusion of oxygen and glucose, and increases oxygenation in ischemic brain tissue. TSC also dampens the intensity of an ischemic challenge during an ongoing ischemic event. The current study examined the impact of TSC in rat models of ischemic and hemorrhagic stroke. Rat three vessel occlusion (3VO), and combined 3VO and one vessel occlusion (3VO/1VO) models of ischemic stroke were evaluated for structural and behavioral outcomes. The effects of TSC were also tested in a rat model of intracerebral hemorrhage (ICH). Delayed treatment with TSC reduced infarct volume in a rodent model of transient focal ischemia involving either 2 or 6 h of ischemia. Neurological outcomes, based on a multi-scale assessment and automated gait analysis, also were improved by TSC treatment. Additionally, TSC reduced edema and hemorrhagic volume in a rat model of ICH. An optimal therapeutic candidate for early intervention in ischemic stroke should be effective when administered on a delayed basis and should not aggravate outcomes associated with hemorrhagic stroke. The current findings demonstrate that delayed TSC treatment improves outcomes in experimental models of both ischemic and hemorrhagic stroke. Together, these findings suggest that TSC may be a safe and beneficial therapeutic modality for early stroke intervention, irrespective of the type of stroke involved.

1. Introduction

Emergency care for stroke must discriminate between ischemic and hemorrhagic strokes in order to safely manage the patient. Tissue plasminogen activator (t-PA) is currently the only FDA-approved therapeutic for the treatment of acute ischemic stroke (NINDs rt-PA STROKE STUDY GROUP, 1995). However, critical limitations to the utility of this therapy exist. First, t-PA

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can aggravate outcomes in hemorrhagic strokes and increase hemorrhagic transformation in some ischemic strokes (NINDs t-PA STROKE STUDY GROUP 1997; (Larrue et al., 2001; Hacke et al., 2004). Second, many patients are ineligible for t-PA treatment because they arrive in emergency departments after the FDA-approved therapeutic window of 3 h, although there remains hope for extending this window (Edlow et al., 2013). Finally, the delay to recanalization after t-PA treatment is highly variable and can be rapid, delayed, complete, partial, or absent (Ribo et al., 2006). Considerable efforts are therefore being directed toward extending the window for t-PA treatment, identifying more effective thrombolytic agents, and limiting the progression of neural injury.

A complementary approach to limiting ischemic injury is to reinstate metabolic supply prior to clot dissolution. During ischemic stroke, areas of partial perfusion can maintain tissue integrity for a few hours and it may be possible to extend this period by increasing the levels of metabolic substrates in the residual flow of blood. Hyperoxic ventilation and/or the administration of compounds that increase the oxygen carrying capacity of blood continue to be tested in this regard (Singhal, 2007). An alternative approach is to enhance the access of metabolic substrates to cells by increasing the diffusion of small molecules into the ischemic tissue. Trans-sodium crocetinate (TSC) is a derivative of the carotenoid crocetin, and can improve the diffusion of oxygen and glucose in aqueous solutions (Laidig et al., 1998; Stennett et al., 2006). Both crocetin and TSC have been shown to improve tissue oxygenation (Seyde et al., 1986; Okonkwo et al., 2003) and enhanced tissue oxygenation can occur without affecting blood flow rates (Holloway and Gainer, 1988). Recent work from our laboratory demonstrated that TSC increases tissue oxygenation in the ischemic penumbra of a rodent model of focal ischemia (Manabe et al., 2010). Moreover, TSC treatment improves structural and behavioral outcomes in animal models of focal cerebral ischemia (Lapchak, 2010a; Manabe et al., 2010). To be considered as a potential therapeutic for stroke, a treatment must be effective when initiated on a delayed basis after the onset of ischemia. Another desirable feature would be a benign or beneficial effect on hemorrhagic stroke outcomes, obviating the need for, and attendant delay associated with, the diagnosis of ischemic versus hemorrhagic strokes. Consequently, the current studies examined the impact of delayed TSC treatment in rat models of both ischemic and hemorrhagic stroke.

2. Results

2.1. *Focal cerebral ischemia*

The effect of TSC was first tested in a model of ischemia–reperfusion involving 2 h of ischemia (3VO) followed by 22 h of reperfusion (Fig. 1). Treatment with TSC ($n=7$) or saline ($n=6$) was initiated 1½ h after the onset of ischemia. The volume of cerebral infarction was significantly reduced by 32% in the TSC-treated group (Fig. 1). No differences in blood gas levels, blood pressure, or rectal temperature were observed between groups during the surgical procedure.

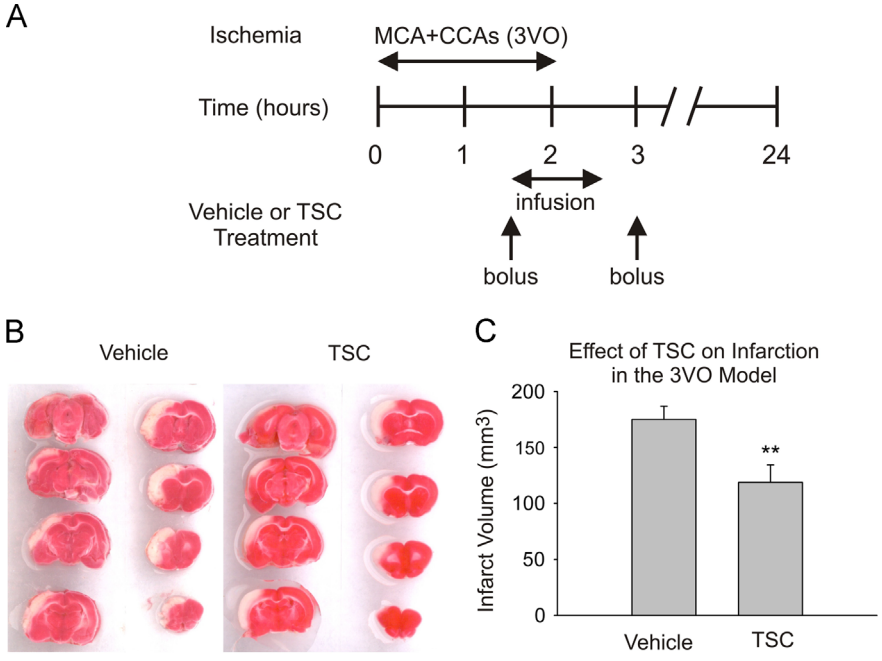

Fig. 1 – Treatment with TSC is protective in a rat protocol of temporary (2 h) focal cerebral ischemia when administered 1½ h after the onset of ischemia. (A) The timeline depicts the period of ischemia (3VO), and the treatment protocol for administering Vehicle or TSC. (B) Serial sections stained with 2,3,5-triphenyltetrazolium chloride (TTC) are shown from the brains of representative animals from the vehicle and TSC groups. The pinkish-red staining represents healthy tissue, while the white areas are regions of infarction. (C) The bar graph presents infarction volumes for the two groups measured at 24 h post-ischemic onset. Values are means and SEMs. The difference in infarct volume between the vehicle Group ($n=6$) and the TSC Group ($n=7$) was statistically significant ($p<0.01$, Student's t-test).**