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Research Report

ERV enhances spatial learning and prevents the development of infarcts, accompanied by upregulated BDNF in the cortex

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ARTICLE INFO

Article history:
Accepted 24 March 2015
Available online 1 April 2015

Keywords:
Neuroprotection
Learning and memory
BDNF
Preclinical study
Neurotrophic factor

ABSTRACT

Purposes: An anti-allergic and analgesic drug, "an extract derived from the inflamed cutaneous tissue of rabbits inoculated with vaccinia virus (ERV)", has been used in medical practice in Japan and some other countries. We examined the effect of ERV, prior to induction of ischemia, on the development of cerebral infarction, on learning and memory, or on brain-derived neurotrophic factor (BDNF) levels in C57BL/6J mice.

Methods: Following oral administration of ERV (the same in humans: × 1) or vehicle, daily for three consecutive weeks, temporary focal ischemia was induced by the three vessel occlusion technique. In the other group of animals, after daily ERV (Low: × 1; Med: × 3, or High dose: × 9) or vehicle administration for three weeks, we performed a quantitative assessment of spatial learning or intracerebral BDNF levels.

Results: The volumes of infarcted lesions, brain edema and the extent of the neurological deficits were significantly reduced in the ERV-treated group. ERV treatment also enhanced spatial learning, accompanied by upregulated BDNF in the cortex.

Conclusions: Daily oral intake of ERV, at a clinically relevant dose, protects the brain from ischemic stroke, and also enhances the learning function in normal mice. As millions of people

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http://dx.doi.org/10.1016/j.brainres.2015.03.042

are currently taking the drug safely, and have been for many years in some cases, there is a need to test the inhibitory actions of the drug on progressive dementia encountered in humans with recurrent ischemic attacks or Alzheimer's disease.

1. Introduction

Ischemic stroke is associated with a progressive reduction in the quality of life and a high mortality rate after onset (Weimar et al., 2010). The cumulative recurrence rate of ischemic stroke was 49.7% at 10 years (Hata et al., 2005), and the cumulative survival rate after stroke, of which 76.5% were ischemic, was 42.8% at 5 years and as low as 24.0% at 10 years (Wolfe et al., 2011). Importantly, more than a third of patients with recurrent stroke episodes demonstrate symptoms related to dementia (Pendlebury and Rothwell, 2009).

The fact that patients with Alzheimer's disease (AD) complicated by brain infarcts had poorer cognitive function and a higher prevalence of dementia than those without infarcts (Snowdon et al., 1997) indicates that cerebral ischemia promotes dementia in AD (Kalaria, 2000). Dementia in AD is associated with accumulation of amyloid β-peptide (Aβ) in the walls of cerebral blood vessels (Bell et al., 2009; Tanzi et al., 2004), and it was found that circulatory disorders in the brain reduce amyloid clearance from cerebral blood vessels into plasma, and that the combination of vascular and neural dysfunction promotes dementia (Kalaria and Ihara, 2013; Dotti and De, 2009; Zaghi et al., 2009). Recently, it was established that impaired cognition is associated with a higher risk of stroke (Rostamian et al., 2014), which also indicates the role of vascular condition in the development of dementia.

For the growing number of people at risk of ischemic stroke and/or cognitive impairment including vascular, aging- or AD-related dementia, the development of a safe compound to protect the brain from ischemic injuries, along with properties that improve brain function (i.e. neurotrophic/neuroprotective properties), is urgently required.

Brain-derived neurotrophic factor (BDNF) is the most abundant growth factor (neurotrophin) in the central nervous system (CNS) with multiple intrinsic regulations and functions (Karpova, 2014; Calabrese et al., 2014; Park and Poo, 2012; Matsumoto et al., 2008). Increased BDNF levels contribute to neuronal survival (Barde, 1989), modulate synaptic consolidation (Soule et al., 2006; Poo, 2001), enhance working memory and spatial learning (Nakajo et al., 2008), improve impaired cognition in Alzheimer's disease (Nagahara et al., 2009), improve symptoms related to depression (Duclot and Kabbaj, 2015; Cai et al., 2015), and also enhance the survival of endothelial cells (Sopova et al., 2014; Kermani and Hempstead, 2007). Enhanced BDNF production in the brain induced by spreading depolarizations (Yanamoto et al., 2008, 1998), or prolonged infusion of BDNF into the brain (Yanamoto et al., 2000), when performed prior to ischemia, prevents the development of cerebral infarction. Conversely, a genetic decrease in BDNF levels causes obesity (Lu et al., 2009) and metabolic syndrome, and decreases longevity (Unger et al., 2007).

Variola virus, with a narrow host specificity, causes smallpox in humans. Vaccinia virus with a broad host range, shares a common antigen with the variola virus, and relatively benign in nature, had been used to generate variola vaccine. Since 1950, "an extract derived from the inflamed cutaneous tissue of rabbits inoculated with vaccinia virus (ERV)" (Neurotropin®) has been used in medical practice widely in Japan and China as an anti-allergic and analgesic drug in humans (Thiebauld et al., 1990; Hutsebaut and De, 1989; Kita et al., 1976; Kita et al., 1975). Following a latency period of some weeks, ERV, a set of biological molecules generated in the cutaneous tissue, consisting of non-protein, low (<5000) molecular weight molecules, relieves chronic pain caused by peripheral neuropathy, neuritis or myopathy, by accelerating the descending inhibitory, anti-nociceptive pathway, probably via increasing gamma-aminobutyric acid (GABA)-ergic synapses, with a record of infrequent and benign side effects (Kudo et al., 2011; Okazaki et al., 2008; Ohara et al., 1991).

Recently, administration of ERV was found to enhance BDNF expression in a human neuroblastoma cell line (Fukuda et al., 2010). ERV also facilitated the time course of tropomyosin-related kinase (trk) auto-phosphorylation, accompanied by the accumulation of Trk-ganglioside GM1 complexes (Fukuda et al., 2015). After the appearance of memory disturbance caused by the accumulation of amyloid β peptide in the cortex and basal ganglia and progressive loss of hippocampal BDNF, repeated oral administration of ERV (200 U/kg/day for three months) prevented the decline in hippocampal BDNF and improved performance in a memory task in Ts65Dn mice (Fukuda et al., 2010).

On the basis of these ERV findings, we investigated the effect of prophylactic or post hoc oral treatment with ERV on the development of cerebral injuries and dysfunction after focal ischemia in mice by analyzing the size of cerebral infarction and the levels of neurological impairment. We also studied the effect of chronic ERV treatment on intra-cerebral BDNF levels, and on the spatial learning and memory function in normal mice.

2. Results

2.1. *The physiological parameters*

There were no significant differences in body temperature, heart rate and mean arterial blood pressure between vehicle and ERV-treated groups during and after ischemia (Table 1).

At the end of the 21-day vehicle or ERV treatment, there was no significant difference in body weight or blood glucose levels [blood glucose: 170±13 mg/dl in vehicle, 166±201, 164±10, or