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Brain Research

Research Report

Therapeutic potential of treatment with the flavonoid rutin after cortical focal ischemia in rats

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ARTICLE INFO

Article history:
Accepted 22 January 2013
Available online 29 January 2013

Keywords:
Stroke
Sensorimotor cortex
Neurodegeneration
Functional recovery

ABSTRACT

Flavonoids have known anti-inflammatory and antioxidative actions, and they have been described as neuroprotective and able to reduce damage in CNS diseases. We evaluated the action of the flavonoid rutin in an animal model of focal cortical ischemia induced by unilateral thermocoagulation of superficial blood vessels of motor (M1) and somatosensory (S1) primary cortices. Ischemic rats were submitted to daily injections (i.p.) for five days, starting immediately after induction of ischemia. We tested two doses: 50 mg/kg or 100 mg/kg of body weight. Sensorimotor tests were used to evaluate functional recovery. Bioavailability in plasma was done by chromatographic analysis. The effect of treatment in lesion volume and neurodegeneration was evaluated 48 h and 72 h after ischemia, respectively. We observed significant sensorimotor recovery induced by rutin, and the dose of 50 mg/kg had more pronounced effect. Thus, this dose was used in further analyses. Plasma availability of rutin was detected from 2 h to at least 8 h after ischemia. The treatment did not result in reduction of lesion volume but reduced the number of degenerated neurons at the periphery of the lesion. The results suggest rutin as an efficient drug to treat brain ischemia since it was able to promote significant recovery of sensorimotor loss, which was correlated to the reduction of neurodegeneration in the periphery of cortical injury. Increasing studies with rutin and other flavonoids might give support for further clinical trials with these drugs.

1. Introduction

Stroke is currently a critical public health problem and a major cause of death and disability in adults worldwide (Lloyd-Jones et al., 2009; Lotufo, 2005). Several pathophysiological events are triggered in brain tissue after an ischemic injury, including the inflammatory response and oxidative stress damage (Brouns and De Deyn, 2009; Deb et al., 2010). Thus, drugs with anti-inflammatory and antioxidative actions have been expected to have a protective effect in brain ischemia.

Abbreviations: ANOVA, Analysis of variance; FJC, Fluoro-Jade C; HPLC, High performance liquid chromatography; i.p., Intraperitoneal; MCAO, Middle cerebral artery occlusion; PID, Post-ischemic day; SEM, Standard error mean; TTC, 2,3,5-Triphenyltetrazolium chloride

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0006-8993
http://dx.doi.org/10.1016/j.brainres.2013.01.039

Polyphenols are natural substances found in plant products, as leaves and fruits, oils, wine and tea. They are divided into phenolic acids, flavonoids and non-flavonoid polyphenols (Ramassamy, 2006). Like beta-carotene and ascorbic acid, polyphenolic compounds are related to protective effects against cancer and cardiovascular disease (Heim et al., 2002). Flavonoids are part of this large group of polyphenolic compounds, and more than 2000 flavonoids have been identified (Ramassamy, 2006). The most important pharmacological properties of flavonoids are its anti-inflammatory and antioxidative actions (Benavente-García and Castillo, 2008; Formica and Regelson, 1995; Juurlink and Paterson, 1998; Procházková et al., 2011). The use of flavonoids has been proposed for pathologies of central nervous system, such as Parkinson's disease, Alzheimer's disease and stroke, due to such properties and to data from epidemiological studies (Ramassamy, 2006; Sun et al., 2008).

Rutin, also called as quercetin-3-O-rutinoside, is a flavonoid glycoside composed of the flavonoid quercetin and the disaccharide rutinose that have antioxidative, anti-inflammatory, antiallergic, anti-viral and anti-carcinogenic actions (Araújo et al., 2011). Few studies have evaluated the treatment with rutin in models of global and focal brain ischemia, showing positive effects (Gupta et al., 2003; Khan et al., 2009). Rutin administration has been evaluated in a model of focal brain ischemia, revealing protective action (Khan et al., 2009). However, only pre-ischemic administration was assessed (Khan et al., 2009).

Here, we studied the effect of treatment with rutin after induction of focal cortical ischemia. Thus, we aimed to analyze whether this flavonoid could be used as medicine to treat brain ischemia. We applied rutin into the acute phase of ischemia and evaluated its bioavailability and its effects on sensorimotor recovery and neurodegeneration.

2. Results

2.1. *Behavioral analyses*

To evaluate whether the administration of rutin after induction of cortical ischemia results in any functional recovery, ischemic animals were treated with rutin and their sensorimotor performance was measured.

In cylinder test, statistical analysis showed significant "treatment x day" interaction ($F=1.56$, $p<0.05$) and significant effects of treatment ($F=3.61$, $p<0.05$) and day ($F=16.5$, $p<0.0001$). Comparisons among groups showed more marked recovery in R50 group, and R100 showed discrete effect (Fig. 1). Thus, rutin promoted significant recovery of contralateral forelimb performance in support during vertical exploration. Similarly, in adhesive test, statistical analysis showed significant "treatment x day" interaction ($F=1.64$, $p<0.05$) and significant effects of treatment ($F=5.18$, $p<0.05$) and day ($F=30.19$, $p<0.0001$). Comparisons among groups also showed more marked recovery in R50 group than in R100 group (Fig. 2). Sham animals were also evaluated and showed no significant lost of function (Fig. 2). Thus, rutin promoted significant recovery of adhesive removal with contralateral forelimb after tactile stimulation.

Together, these results suggest that post-ischemic treatment with rutin is effective to recover sensorimotor function after cortical focal ischemia. Since the dose of

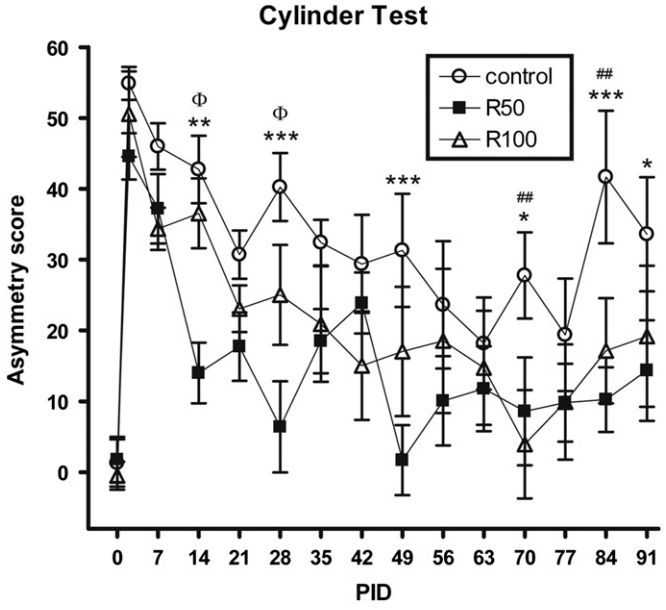

Fig. 1 – Recovery of the impaired forelimb in the cylinder test. Graph showing the accompaniment of the performance of control ($n=7$), R50 ($n=6$) and R100 ($n=7$) groups before ischemia and along post-ischemic weeks. In all groups, the greater asymmetry was observed at PID 2. R50 group was significantly different from the control group at PIDs 14, 28, 49, 70, 84 and 91, showing a higher level of recovery. R100 group showed recovery only at PIDs 70 and 84. * represents the comparison between control and R50 groups, # represents the comparison between control and R100 groups and Φ represents the comparison between R50 and R100 groups. (* or Φ=$p<0.05$; ##=$p<0.01$; ***=$p<0.001$; Tukey).