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Research Report

Pinocembrin protects rat brain against oxidation and apoptosis induced by ischemia–reperfusion both in vivo and in vitro

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ABSTRACT

Pinocembrin is one of the flavonoids at the highest concentration in propolis. In this study, we investigated the neuroprotective effect of pinocembrin on ischemia/reperfusion and ischemia/reperfusion-like insults. Protection by pinocembrin was studied at the in vivo level using a model of middle cerebral artery occlusion and reperfusion in rats. Pinocembrin was administrated at the start of reperfusion. Pinocembrin markedly increased rat viability, reduced infarct volumes and neurological deficit scores in all treatment groups. Primary cortical neuronal cultures were subjected to oxygen–glucose deprivation/reoxygenation, a model of ischemia/reperfusion-like injury, and treated with pinocembrin at the start of reoxygenation. Neuronal survival rates were increased, LDH release was decreased and both neurite length and apoptosis were alleviated when pinocembrin was present during reoxygenation, and this protection was associated with the reduction of reactive oxygen species, nitric oxide and neuronal nitric oxide synthase (nNOS) and inducible NOS (iNOS), and an increase of glutathione. Moreover, DNA laddering was decreased in treatment groups of pinocembrin. Caspase-3 protein was down-regulated and PARP degradation was alleviated after pinocembrin treatments. Our results suggest that pinocembrin may be a novel therapeutic strategy to reduce cerebral ischemia/reperfusion injury, and may act by the anti-oxidative and anti-apoptotic effects.

1. Introduction

Pinocembrin (5, 7-dihydroxyflavanone, Fig. 1), a natural compound, is one of the flavonoids at the highest concentration in propolis. As flavonoids with a broad spectrum of pharmacological activities, not a few of biological actions of pinocembrin have been reported since 1980s, including anti-microbial (Pepeljnjak et al., 1985; Del Rayo Camacho et al., 1991), anti-oxidant (Santos et al., 1998), anti-inflammatory (Sala et al., 2003), and endothelium-relaxation effects (Zhu et al., 2007). In recent years, many new researches in our laboratory on this compound were undertaken, and some findings indicated that pinocembrin has some protective effects on ischemic injury. For example, one of our previous studies has shown that it could improve rat cognitive impairments induced by chronic cerebral hypoperfusion by the contribution to its protections on brain mitochondria structure and function (Guang and Du, 2006). However, there was no report on the effects of pinocembrin on brain injured after focal cerebral ischemia followed by reperfusion, and the mechanisms of its neuroprotective effects remain poorly understood.

As we know, cerebral ischemia/reperfusion (I/R) injury has been becoming more and more important with the

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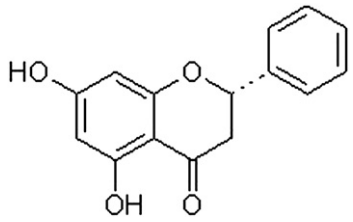

Fig. 1 – Structure of pinocembrin.

development of thrombolytic drugs. Molecular mechanisms of neuronal cytotoxicity following ischemia/hypoxia have been furthered considerably in the last several years, yet many questions remain as to the precise pathways of injury and consequently the best strategies to prevent or interrupt the cascade of excitotoxicity and death (Mishra, 1999). Oxygen free radicals are likely participants in the pathogenesis of cerebral I/R. Recent studies have provided direct and indirect experimental evidence showing that oxygen free radicals are elevated during I/R because of the failure of metabolic reactions (Love, 1999). Moreover, anti-oxidant defenses including free radical scavengers and anti-oxidant enzymes are considered a promising approach to limit the extent of damage of I/R injury (Papadopoulos et al., 1998; Truelove et al., 1994). Therefore, in the present study, the neuroprotective effect of pinocembrin was evaluated both in a MCAO–reperfusion rat model, the primary site of many strokes, which is considered to more closely mimic the clinical situation (Macrae, 1992) and an oxygen–glucose deprivation/reoxygenation of primary cultured cortical neurons, a model of ischemia/reperfusion-like injury. Its neuroprotective mechanism against cerebral ischemia/reperfusion was further studied.

2. Results

2.1. *Effects of pinocembrin on viability, neurological deficit scores and infarct volumes in rats subjected to 2 h of middle cerebral artery occlusion and 24 h of reperfusion*

The results showed that pinocembrin treatment increased rat viability and reduced neurological deficit scores and infarct volume in transient focal ischemic rats. As shown in Table 1 and Fig. 2, neurological deficit scores and infarct volumes of MCAO/reperfusion rats were significantly higher than those of the sham-operated group after 24 h reperfusion. Treatment with pinocembrin (3, 10, 30 mg/kg, i.v.) significantly reduced the percentage of infarction in the ipsilateral hemisphere to 24.08%, 20.87%, and 28.83%, respectively, and decreased neurological deficit scores as well. The results demonstrated that pinocembrin protected rat brain from ischemia–reperfusion damage.

2.2. *Effects of pinocembrin on primary cultured cortical neurons*

2.2.1. *Effects of pinocembrin on the morphological changes of OGD/reoxygenation injured cortical neurons*

Neuronal morphology was examined after the exposure to 2 h-OGD/24 h-reoxygenation at 37 °C and neuronal morphological changes were observed by both optics and fluorescent imaging. OGD/reoxygenation induced neuronal loss and the damage of neurites. Some neurons showed morphological features of necrotic cells, including swelling, loss of plasma membrane integrity and eventual dissolution of the cell. Meanwhile, during the progression of cellular necrosis, some neurons displayed condensed cytoplasm, cell shrinkage with the plasma membrane remaining intact and formation of apoptotic bodies that stained more densely with Hoechst 33258. In the presence of pinocembrin, the exposure to OGD/reoxygenation produced less neuronal degeneration and loss. The neuronal network and neuronal cell bodies were partly preserved.

As shown in Figs. 3 and 4, OGD/reoxygenation induced the damage of neurites. Relative to control group, the neurite length had significant changes in OGD/reoxygenation groups, decreased to 102.72±29.0632 μm ($^{*}P<0.01$). Compared with OGD/reoxygenation group, pinocembrin treatments after OGD increased neurite length to 209.79±37.2603 μm, 106.88±25.8613 μm, and 135.53±25.7985 μm ($n=6$, $^{\#}P<0.01$, $^{\&}P<0.05$), respectively. The value of a high concentration of pinocembrin treatment increasing neurite length was more obvious than those of middle and low concentrations ($^{@}P<0.05$).

2.2.2. *Effects of pinocembrin on the viability of OGD/reoxygenation injured cortical neurons*

As shown in Fig. 5, OGD/reoxygenation appeared to induce neurotoxicity. Relative to control group, neuron survival rate had a significant change in OGD/reoxygenation group, decreased to 49.34±7.95% ($n=6$, $P<0.01$) after 24 h-reoxygenation. Compared with OGD/reoxygenation group, pinocembrin treatments increased neuron survival rates to 85.11±13.07%, 77.39±19.62% and 70.61±10.40% ($n=6$, $P<0.05$) respectively. There was no significant change between the values of high, middle and low concentrations of pinocembrin treatments in the measurement of neuronal viability.

Table 1 – Effects of pinocembrin on viability, neurological deficit scores and infarct volumes in rats subjected to 2 h of middle cerebral artery occlusion and 24 h of reperfusion

Model	Dose (mg/kg)	Viability	Neurological deficit scores	Infarction volumes
Sham	3 mg/kg, NS	100%	0.0±0.0	0.0±0.0
Model	3 mg/kg, NS	45.45%	3.2±0.68*	41.24%±1.61%*
DL0108	3	83.33%	2.0±0.58#	24.08%±2.32%#&
DL0108	10	66.67%	1.75±0.39#	20.87%±2.32%#&
DL0108	30	83.33%	2.4±0.45#	28.83%±3.28%#

Animals received pinocembrin (3, 10, 30 mg/kg, i.v.) or vehicle at the beginning of reperfusion. After 24 h of reperfusion, animal viability, neurological deficit scores and infarction volumes were detected as described. Total *N* used in each group is 6 in sham and pinocembrin (3, 10, 30 mg/kg) groups, and 11 in model group. Data were presented as mean±S.D. of three independent experiments. Data of viability and infarct volumes were analyzed by one-way ANOVA, and data of neurological deficit scores were analyzed by Wilcoxon rank-sum test. NS means normal sodium. Differences were considered significant at $P<0.05$. $^{*}P<0.05$ vs. sham-operated group. $^{*}P<0.01$ vs sham. $^{\#}P<0.05$ vs. model group. $^{\&}P<0.05$ vs. pinocembrin 30 mg/kg group.