ELSEVIER

Toxicon 46 (2005) 845–851

TOXICON

www.elsevier.com/locate/toxicon

Catalpol prevents the loss of CA1 hippocampal neurons and reduces working errors in gerbils after ischemia-reperfusion injury

Dan-Qing Li[a,b], Yang Li[a], Yuxin Liu[a], Yong-Ming Bao[a], Bo Hu[c], Li-Jia An[a,*]

[a]*Department of Bioengineering, School of Environment and Life, Dalian University of Technology, Dalian, Liaoning 116023, People's Republic of China*

[b]*The Second Affiliated Hospital, Dalian Medical University, Dalian, Liaoning 116023, People's Republic of China*

[c]*Department of Pathology, The Attached Hospital, Dalian University, Dalian, Liaoning 116023, People's Republic of China*

Available online 2 November 2005

Abstract

Catalpol, an iridoid glycoside, contained richly in the roots of *Rehmannia glutinosa*, was found for the first time to be of neuroprotection in gerbils subjected to transient global cerebral ischemia. Catalpol (1 mg/kg ip) used immediately after reperfusion and repeatedly at 12, 24, 48 and 72 h significantly rescued neurons in hippocampal CA1 subfield and reduced working errors during behavioral testing. The neuroprotective efficacy of catalpol became more evident when the doses of catalpol were increased to 5 and 10 mg/kg. In addition, it was exciting that the significant neuroprotection by catalpol was also evident when catalpol was applied up to 3 h after ischemia. But the neuroprotective efficacy of catalpol became weak when catalpol was given at 6 h after ischemia. Of great encouragement was the finding that the neuroprotection of catalpol could be seen not only in a short post-ischemic period (12 days) but also in a long period (35 days). All these indicated that catalpol was truly neuroprotective rather than simply delayed the onset of neuronal damage and might be of therapeutic value for the treatment of global cerebral ischemia.

Keywords: Catalpol; Ischemia; Gerbils; Hippocampus; Cognition; Neuroprotection

1. Introduction

Hippocampus is the most vulnerable region to ischemia, it is also an important area to critically involve in learning and memory processes. Transient global cerebral ischemia can induce delayed neuronal death (DND) in the hippocampus in both humans and other animals (Kirino, 1982; Petito et al., 1987) and further lead to cognitive impairment including learning disability and amnesic behavior (Catania et al., 2002), which has been widely evaluated in gerbils (Iqbal et al., 2002; Sala et al., 1997; Andersen and Sams-Dodd, 1997). DND is often regarded as apoptosis because the molecular biological and biochemical as well as morphological characteristics of apoptotic cell death have been detected in DND. Although the mechanisms are unclear completely, many factors such as glutamate release, excitotoxicity, Ca^{2+} overload, oxygen stress and inflammation, etc. have been suggested to be associated with DND. A great number of compounds targeting these specific pathways have been proven to be effective in animal ischemic models. However, there is no agent the efficacy of which has been established in human up to now. One of the most important reasons is a relatively low dosage of drugs in human trials compared to the doses used in animal experiments due to unbearable side effects observed in

* Corresponding author. Tel.: +86 411 84706356; fax: +86 411 84706365.

E-mail address: bioeng@dlut.edu.cn (L.-J. An).

0041-0101/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2004.09.007

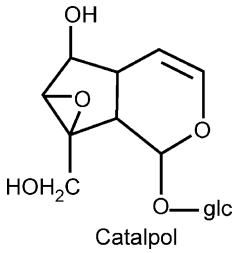

Fig. 1. The structure of catalpol.

higher doses. In light of this viewpoint, there is an increasing interest in focusing on natural products now. Some natural products separated from traditional Chinese herbal medicine have been shown to be of neuroprotection in vitro in our laboratory (Jiang et al., 2003, 2004; Yu et al., 2003).

Iridoid glycoside is a kind of iridoids and exists broadly in plants of many families and has a wide variety of biological activities including purgative, liver protective, anti-microbial, analgesic, anti-tumor, sedative and anti-inflammatory activities (Ismailoglu et al., 2002), but its neuroprotective effect to our knowledge has been scarcely studied. Catalpol (Fig. 1), extracted from the roots of *Rehmannia glutinosa*, is also an iridoid glycoside. Recently, it was shown to attenuate apoptosis induced by H_2O_2 in PC12 cells in vitro (Jiang et al., 2004). It is generally believed that ischemia-reperfusion is accompanied by the increment of hydrogen peroxide. According to these findings, we guess that catalpol may be of neuroprotection in cerebral ischemia. To our knowledge, there is no previous study on the neuroprotective effects of catalpol against neuronal damage after focal or global cerebral ischemia in vivo. In this study, Mongolian gerbils were used because of their deficiency in cerebral blood flow between the posterior arteries and the common carotid arteries. Occluding the bilateral common carotid arteries temporarily is sufficient to induce DND in hippocampal CA1 subfield. Therefore, transient global cerebral ischemia in Mongolian gerbils is a useful animal model for screening neuroprotective drugs. It was encouraging to find in gerbil ischemic model that catalpol could prevent the loss of hippocampal CA1 neurons and reduce working errors dose-dependently. The neuroprotection of catalpol could be seen not only in a short post-ischemic period but also in a long period, more exciting was that the neuroprotection still excellently kept even when the using of catalpol was delayed for 3 h after ischemia.

2. Materials and methods

2.1. Chemical and chemical doses

Catalpol was of analytical grade (purity $>98\%$) and purchased from National Institute for The Control of Pharmaceutical and Biological Products and dissolved in physiological saline. Catalpol was administered to normal gerbils (four in each group) at 100, 50, 20, 10 and 5 mg/kg by intra-peritoneal injection, and the effects of the drug on gerbils' behavior were observed. At 100 and 50 mg/kg, mortality rate was 100%. At the dose of 20 mg/kg, mortality rate was 25%. The gerbils that survived suffered from paralysis. At 10 and 5 mg/kg, the gerbils did not show any abnormality. Therefore, the doses below 10 mg/kg were chosen in this study.

2.2. Animals

Mongolian gerbils (obtained from Experimental Animal center, Dalian Medical university, China), half male and half female, aged 12 weeks and weighing 50–70 g, were housed in cages in an air-conditioned room with controlled temperature (24 ± 1 °C) for 5 days before the experiment and were maintained on a 12 h:12 h light cycle (07:00 on–19:00 off). They were allowed free access to food and water. All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Dalian Medical University, Dalian, China.

2.3. Induction of ischemia

Transient global ischemia was induced by bilateral occlusion of the common carotid arteries (CCA) (Wang et al., 2002). Briefly, gerbils were anesthetized with diethyl ether and then fixed in supine position. A midline incision was made in the ventral neck. Both CCA were separated carefully and occluded simultaneously for 5 min with non-traumatic micro-aneurysm clips. At the end of the ischemic period, the clips were removed to allow cerebral reperfusion. Blood flow during the occlusion and reperfusion after the removal of the clips was confirmed by direct visualization. Then the neck incision was closed with 4–0 silk sutures. Sham-operated animals underwent the identical procedures except clips were not applied. The onset of cerebral ischemia was associated with a brief period of panting breathing and body movements followed by quiescence (Candelario-Jalil et al., 2002). Only animals showing these behaviors were considered in this study. The rectal temperature of the animals was carefully monitored and maintained at 37.0–37.5 °C throughout the surgery using an incandescent lamp. Animals were returned to their home cages after recovery from the surgery.

2.4. Catalpol treatment protocols

2.4.1. Experiment 1

This experiment was undertaken to investigate the efficacy of three different doses of catalpol in protecting against the loss of CA1 neurons and cognitive impairment in gerbils after ischemic injury. Five experimental groups were designed as a sham-operated group, an ischemic group