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SCIENCE DIRECT

Free Radical Biology & Medicine 39 (2005) 495 – 510

www.elsevier.com/locate/freeradbiomed

Original Contribution

Cinnamophilin reduces oxidative damage and protects against transient focal cerebral ischemia in mice

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Received 1 January 2005; revised 25 March 2005; accepted 4 April 2005
Available online 30 April 2005

Abstract

Acute neuroprotective effects of cinnamophilin (CINN; (8*R*, 8′*S*)-4, 4′-dihydroxy-3, 3′-dimethoxy-7-oxo-8, 8′-neolignan), a novel antioxidant and free radical scavenger, were studied in a mouse model of transient middle cerebral artery (MCA) occlusion. CINN was administered intraperitoneally either 15 min before (pretreatment) or 2 h after the onset of MCA occlusion (postischemic treatment). Relative to vehicle-treated controls, animals pretreated with CINN, at 20–80 mg/kg, had significant reductions in brain infarction by 33–46% and improvements in neurobehavioral outcome. Postischemic administration with CINN (80 mg/kg) also significantly reduced brain infarction by 43% and ameliorated neurobehavioral deficits. Additionally, CINN administration significantly attenuated in situ accumulation of superoxide anions (O_2^-) in the boundary zones of infarct at 4 h after reperfusion. Consequently, CINN-treated animals exhibited significantly decreased levels of oxidative damage, as assessed by immunopositive reactions for 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 4-hydroxynonenal (4-HNE), and the resultant inflammatory reactions at 24 h postinsult. It is concluded that CINN effectively reduced brain infarction and improved neurobehavioral outcome following a short-term recovery period after severe transient focal cerebral ischemia in mice. The finding of a decreased extent of reactive oxygen species and oxidative damage observed with CINN treatment highlights that its antioxidant and radical scavenging ability is contributory.

Keywords: Stroke; Focal cerebral ischemia; Neuroprotection; Superoxide; Oxidative damage; Cinnamophilin

Introduction

Oxidative damage has been implicated in various modes of acute brain damage and chronic neurodegeneration, including focal ischemic stroke [1,2]. Ischemia induces an imbalance of endogenous oxidants and antioxidants and overproduction of toxic free radicals [3–6]. Reperfusion also comes with massive production of reactive oxygen (ROS) and nitrogen species (RNS) that potentiates initial brain damage caused by ischemia [3,7]. In particular, superoxide anion (O_2^-), one of the key mediators of excitotoxicity and disturbed Ca^{2+} homeostasis, plays an important role in oxidative chain

Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; CINN, (8*R*, 8'*S*)-4,4′-dihydroxy-3,3′-dimethoxy-7-oxo-8,8′-neolignan; LCBF, local cortical blood flow; MCA, middle cerebral artery; LDF, laser-Doppler flowmetry; HPCD, hydroxypropyl-β-cyclodextrin; 4-HNE, 4-hydroxynonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PBS, phosphate-buffered saline; HEt, hydroethidine.

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doi:10.1016/j.freeradbiomed.2005.04.004

reactions. Accumulations of toxic free radicals, therefore, not only increase the susceptibility of brain tissues to oxidative damage but also trigger various cascades of ischemic injury, leading to either direct injury via membranous lipid peroxidation and protein and DNA oxidation or indirect damage via inflammation and apoptosis [3,5,7]. One strategy, therefore, to protect the brain against ischemic-reperfusion injury is to improve the endogenous antioxidant defense to the tissues "at risk," and/or to decrease oxidative damage by scavenging toxic free radicals which are excessively produced in the ischemic tissues [7–9].

Cinnamophilin (CINN), (8*R*, 8′*S*)-4,4′-dihydroxy-3,3′-dimethoxy-7-oxo-8,8′-neolignan isolated from *Cinnamomum philippinense*, is a novel antioxidant and free radical scavenger [10]. The agent is highly lipophilic and, therefore, has the potential to cross the blood-brain barrier to the brain. It is a dual inhibitor of thromboxane synthase and thromboxane A2 receptor [11,12], and has the ability to block Na^+ and Ca^{2+} inward currents in rat cardiac cells [13]. CINN has also been demonstrated to protect against ischemic-reperfusion injury of muscle in vivo [14]. More recently, CINN has been shown to concentration—dependently suppress the non-enzymatic iron-induced lipid peroxidation and copper-catalyzed oxidation and the activity on NADPH-dependent microsomal lipid peroxidation in rat brain homogenates [10]. Accordingly, we supposed that CINN could protect against cerebral ischemia-reperfusion in vivo.

In the present study, we therefore examined whether pretreatment with CINN (20-80 mg/kg) would reduce poststroke brain damage and improve functional outcome following transient cerebral ischemia in C57/B6 mice and, if it did, further evaluated whether postischemic treatment at the optimal dosage, 2 h after the onset of the ischemic insult, could offer histological and functional neuroprotection. Additionally, we examined spatial and temporal local cortical blood flow (LCBF) changes among CINN-treated animals and controls. Finally, we sought to determine whether CINN treatment could reduce postischemic accumulations of ROS and, consequently, decrease membrane lipid peroxidation, DNA hydroxylation, and postischemic inflammation in the ischemic brain.

Materials and methods

All procedures performed were approved by the Subcommittee on Research Animal Care of the University Medical Center, whose standards meet the guidelines of the National Institutes of Health (*Guide for the Care and Use of Laboratory Animals*).

Animal preparation and anesthesia

Adult male C57 black (C57BL/B6) mice, weighing 20–27 g, were supplied by the University Laboratory Animal Center, and were allowed free access to food and water before and after surgery. Animals were anesthetized with 1% halothane in 70% N_2O/30% O_2. During surgery, body temperature was maintained at 37 ± 1°C using a heating lamp and a thermostatically controlled heating blanket and rectal probe (Harvard Apparatus, South Natick, MA).

Experimental model, drug administration, and grouping of animal

Focal cerebral ischemia was employed by intraarterial suture occlusion of the proximal right middle cerebral artery (MCA) [9,15,16]. Briefly, the bifurcation of the right common carotid artery was exposed under an operating microscope. A 6-0 nylon suture, with its tip rounded by heating over a flame and subsequently coated with silicone (Merck KGaA, Darmstadt, Germany), was advanced 7.5–8.5 mm from the external into the internal carotid artery until the tip occluded the origin of the MCA. After closure of the operative sites, the animals were allowed to awaken from the anesthesia and temporarily transferred to a cage with a heating lamp (ambient temperature ≈26 ± 1°C). During another brief period of anesthesia, the suture was gently removed at 60 min of MCA occlusion. Reperfusion was ensured by an improvement in the ipsilateral LCBF at a defined area of the ischemic core cortex to about 50% of baseline following an initial decrease to about 12% of baseline caused by MCA occlusion as determined by laser-Doppler flowmetry (LDF, Laserflo BMP^2, Vasamedics, St. Paul, MN) [9,17,18]. After the surgical procedures, the animals were kept in a cage with a heating lamp and monitored for 4 h and then transferred into the home cage (ambient temperature ≈24 ± 1°C).

Drug administration and grouping of animals for acute neuroprotective testing

CINN (molecular structure shown in Fig. 1) was isolated from the root of *C. philippinense* as previously described [11,12] and dissolved in 45% aqueous hydroxypropyl-β-cyclodextrin (HPCD; Sigma Chemical Co., St Louis, MO). Fresh drug solution was prepared shortly before its administration. Throughout the whole course of experiments, animals were randomly assigned to each treatment

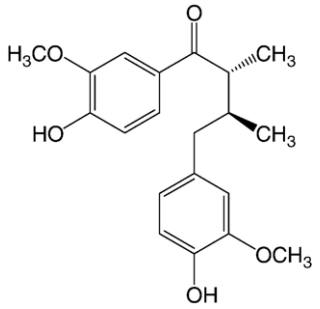

Fig. 1. Structure of cinnamophilin (CINN, $C_{20}H_{24}O_5$). The molecular weight is 344.