Pharmacological Reports 67 (2015) 115–122

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Pinocembrin attenuates hippocampal inflammation, oxidative perturbations and apoptosis in a rat model of global cerebral ischemia reperfusion

Muhammed A. Saad *, Rania M. Abdel Salam, Sanaa A. Kenawy, Amina S. Attia

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 24 June 2014
Received in revised form 13 August 2014
Accepted 14 August 2014
Available online 27 August 2014

Keywords:
Pinocembrin
Ischemia/reperfusion
Oxidative stress
Apoptosis
Inflammation

ABSTRACT

Background: Pinocembrin is a major flavonoid molecule isolated from honey and propolis. It has versatile pharmacological and biological activities including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities as well as neuroprotective effects against cerebral ischemic injury. The purpose of the current study was to determine the possible mechanisms of neuroprotection elicited by pinocembrin with specific emphasis on chronic prophylactic use before the induction of global cerebral ischemia reperfusion.

Methods: Global cerebral ischemia–reperfusion (I/R) was induced by bilateral carotid artery occlusion for 15 min followed by 60 min reperfusion period. Animals were randomly allocated into 3 groups ($n = 28$): Sham operated, I/R control and rats treated with pinocembrin (10 mg/kg, *po*) daily for 7 days then I/R was induced 1 h after the last dose of pinocembrin. After reperfusion rats were killed by decapitation, brains were removed and both hippocampi separated and the following biochemical parameters were estimated; lactate dehydrogenase activity, oxidative stress markers (lipid peroxides, nitric oxide and reduced glutathione), inflammatory markers (myeloperoxidase, tumor necrosis factor-alpha, nuclear factor kappa-B, interleukin-6 and interleukin-10), apoptotic biomarkers (caspase 3 and cytochrome C), neurotransmitters (glutamate, gamma aminobutyric acid) and infarct size were assessed.

Results: Pinocembrin ameliorated damage induced by I/R through suppressing oxidative stress, inflammatory and apoptotic markers as well as mitigating glutamate and lactate dehydrogenase activity. One of the more significant findings to emerge from this study is that pinocembrin normalized the infarct size elevated by I/R.

Conclusions: Pinocembrin showed a neuroprotective effects through antioxidant, anti-inflammatory and antiapoptotic mechanisms.

Introduction

Pinocembrin, one of the most important flavonoids isolated from honey and propolis. It possesses anti-inflammatory, antimicrobial, and antioxidant activities [1–3]. The extensive research on pinocembrin indicated that it has potential biological activities, which have made a further interest in studying its activities in many other disorder of global concern. Stroke is one of these disorders as it is considered the leading cause of morbidity and mortality world-wide [4]. Acute brain ischemia–reperfusion (I/R) injury is the major pathophysiological sign of ischemic stroke [5].

Pinocembrin showed antiapoptotic effects by attenuating endoplasmic reticulum stress in a model of focal and global (I/R) [6,7]. Furthermore, it decreased neurological score, brain edema and concentrations of Evan's blue (EB) and fluorescein sodium in brain tissue suggesting an alleviation in blood–brain barrier injury induced by global cerebral I/R [8,9]. Additionally, pinocembrin has proven to be a possible neuroprotective agent against global

Abbreviations: EB, Evan's blue; ELISA, enzyme-linked immunosorbent assay; GSH, reduced glutathione; HPLC, high performance liquid chromatography; I/R, ischemia/reperfusion; IFN, interferon; IL-6, interleukin-6; IL-10, interleukin-10; LDH, lactate dehydrogenase; MPO, myeloperoxidase; NF-κB, nuclear factor kappa B; NO_x, *nitric* oxide; SO, Sham operated.; TBARs, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-alpha..

* Corresponding author.
E-mail address: muhammed.abdullatif@gmail.com (M.A. Saad).

http://dx.doi.org/10.1016/j.pharep.2014.08.014
1734-1140/

cerebral I/R injury through its antioxidant, anti-excitotoxic and anti-inflammatory effects [6,10]. Wang et al. [11] suggested that inhibiting soluble epoxide hydrolase and then increasing the potency of epoxyeicosatrienoic acids may be one of the mechanisms through which pinocembrin provides cerebral protection. Another possible mechanism for neuroprotection may be through anti-inflammatory actions as evidenced by the reduced expressions of interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and inducible NO synthase shown by pinocembrin [12].

So far, there has been considerable research on the curative effect of pinocembrin if given on acute basis, however, there has been little discussion about the prophylactic effect of pinocembrin if given chronically before induction of I/R. Therefore, it deemed of importance to study the prophylactic effect of pinocembrin if given on chronic basis. Although extensive research has been carried out on pinocembrin, no single study exists which adequately covers its effect on infarct size, neurotransmitter as GABA and glutamate and nuclear factor kappa-B (NF-κB). Furthermore, few writers have been able to draw on any systematic research into the full mechanism of neuroprotection induced by pinocembrin against damage induced by I/R. Hence the current investigation were aimed to study the prophylactic effect of pinocembrin against injury induced by global cerebral I/R if given chronically for seven days before the induction of I/R. The central question in this dissertation asks how pinocembrin exerts its neuroprotective effect, and was answered through making a full investigation of the possible mechanisms of its neuroprotection by studying the changes in oxidative stress, inflammatory and apoptotic biomarkers as well as neurotransmitters and infarct size.

Materials and methods

Animals

Male Wistar rats weighing; 250–300 g were obtained from the National Scientific Research Center (Giza, Egypt). Animals were housed for at least one week in the laboratory room prior to testing. They were kept under controlled environmental conditions; room temperature (24–27 °C), constant humidity (60 ± 10%), with alternating 12 h light and dark cycles. Food (standard pellet diet) & water were allowed *ad libitum*. The Ethics Committee of Faculty of Pharmacy Cairo University approved this study. All animals' procedures were performed in accordance to the institutional Ethics Committee and in accordance with the recommendations for the proper care and use of laboratory animals. Unnecessary disturbance of animals was avoided. Animals were treated gently; squeezing, pressure and tough maneuver were avoided.

Global cerebral ischemia and experimental material preparation

Animals were randomly allocated into 4 groups (n = 28 rats per group) as follows:

Group I: Sham operated (SO).
Group II: ischemia–reperfusion group (I/R).
Group III: pinocembrin pretreatment; rats were given pinocembrin (10 mg/kg, *po*) daily for 7 days [13], then I/R was induced 1 h after the last dose of pinocembrin.

Each group was subdivided into 2 subsets. The first subset (n = 24 rats) was used for biochemical estimations, while the second subset (n = 4 rats) served for measurement of infarction size.

In all groups rats were anaesthetized with thiopental (50 mg/kg, *ip*) and midline ventral incision was made in the neck. Bilateral carotid artery occlusion using small artery clips was done to induce global cerebral ischemia for 15 min followed by 60 min reperfusion period except for the SO group in which the artries were exposed for 75 min without occlusion. After reperfusion rats were euthanized by cervical dislocation. Brains were removed immediately and both hippocampi were dissected on ice cold plates. The first subset (n = 24 rats) were subdivided into 2 sets, in one set of animals (n = 16 rats), the hippocampi were homogenized in isotonic ice cold saline using a glass homogenizer (HeidolphDiax 900, Germany). This homogenate was used for the determination of NO_x, TBARs, GSH, IL-10, IL-6, NFκB and TNF-α contents as well as Cytochrome C and Caspase 3 activities. An aliquot of 60 μl of this homogenate was centrifuged at 105,000 × g for 45 min at 4 °C using cooling ultra-centrifuge (Sorvall-Compiplus T-880, Dupont, USA) to separate the cytosolic hippocampal fraction, which was used for determination of the activity of LDH. In another subset (n = 8 rats), the hippocampi were divided into two portions: one hippocampus was homogenized in hexadecyltrimethylammonium bromide (0.5%) in potassium phosphate buffer (100 mM, pH 6) for the determination of myeloperoxidase (MPO) activity, while the other hippocampus was homogenized in 70% high performance liquid chromatography (HPLC) methanol (1/10, weight/volume) for the determination of neurotransmitter (Glutamate and GABA) contents. All parameters were normalized to protein content, measured according to Lowry et al. [14].

Determination of lactate dehydrogenase (LDH) activity in rat hippocampus

The activity of LDH in rat hippocampus was determined by colorimetric kinetic determination using kit supplied by Biosystems (Biosystems, S.A. Costa Brava, 30 08030 Barcelona, Spain) according to the method of Lorentz et al. [15]. LDH specifically catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH. The rate at which NADH forms is proportional to LDH activity. The method determines the increase in NADH absorbance per minute using a double beam spectrophotometer at wavelength 340 nm.

Determination of lipid peroxides content in rat hippocampus

The lipid peroxidation products are determined by the estimation of the level of thiobarbituric acid reactive substances (TBARs) that are measured as MDA according to the method of Mihara and Uchiyama [16]. MDA is the decomposition product of the process of lipid peroxidation and is used as an indicator of this process.

The principle of the assay depends on a colorimetric determination of a pink pigment product, which results from the reaction of TBARS with thiobarbituric acid in acidic medium at high temperature. To increase the sensitivity of the method, the resultant colored product was extracted in n-butanol and measured at two wavelengths, namely 520 and 535 nm using Shimadzu double beam spectrophotometer (UV-150-02, Japan), to exclude interfering substances. The difference in absorbance at both wavelengths is used to calculate the content of TBARS in the sample.

Determination of reduced glutathione (GSH) content in rat hippocampus

Determination of GSH content in rat hippocampus was performed according to the method of Beutler et al. [17]. The method depends on the fact that both protein and non-protein SH-groups (mainly GSH) react with Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)] (DTNB) to form a stable yellow color of 5-mercapto-2-nitrobenzoic acid, which can be measured at 412 nm. In order to determine the GSH content in tissue, precipitation of