European Journal of Pharmacology 667 (2011) 230–237

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural Pharmacology

Protective effect of Etoricoxib against middle cerebral artery occlusion induced transient focal cerebral ischemia in rats

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ARTICLE INFO

Article history:
Received 19 November 2010
Received in revised form 21 April 2011
Accepted 11 May 2011
Available online 27 May 2011

Keywords:
Cerebral ischemia
Etoricoxib
tMCAO
Oxidative stress
Stroke

ABSTRACT

Stroke is the third leading cause of global death and disability. Cyclooxygenase-2 mRNA has been shown to be up-regulated after stroke and also the time window of its expression extends from 4 to 12 h. The objective of this study was to elucidate the protective effect of Etoricoxib (a selective Cyclooxygenase-2 inhibitor) against transient middle cerebral artery occlusion induced behavioral, biochemical and histological alterations. Transient ischemia reperfusion significantly caused behavioral (neurological deficits, decreased locomotor activity and rotarod performance), biochemical (increased lipid peroxidation and nitrite concentration, while decreased superoxide dismutase and catalase activity) and histological (increased infarct volume) changes. Etoricoxib (3 and 10 mg/kg, i.p.) significantly reversed the alterations caused by cerebral ischemia however, 1 mg/kg dose was not found effective in any of the parameters. Finally, we can conclude that Etoricoxib has beneficial effects against transient middle cerebral artery occlusion model in rats. The present study indicates that Etoricoxib may be considered as a potential candidate in the treatment of stroke, clinically.

1. Introduction

Stroke is the third leading cause of death and disability following cardiovascular disease and cancer. According to a projection by Elkins and Johnston, fatalities due to stroke will increase exponentially in the next 30 years due to aging of the population and our inability to control risk factors (Elkins and Johnston, 2003). Stroke can be ischemic or hemorrhagic with 88% or 12% prevalence, respectively (Rosamond et al., 2008).

Cytokines have been shown to be up-regulated in the brain after a variety of insults including stroke. They are expressed not only in cells of the immune system, but also in resident brain cells including glia and neurons.(Sairanen et al., 2001). Arachidonic acid released from brain phospholipids during ischemia reperfusion is converted to Prostaglandin H_2 by Cyclooxygenase (COX) enzyme. There are two isoforms of COX, of which COX-1 (considered to be a housekeeping gene) is constitutively expressed in many cell types including microglia and leukocytes during brain injury (Schwab et al., 2002). COX-2, which was isolated as an inducible immediate-early gene, is believed to play a negative role in brain injury including ischemia (Collaco-Moraes et al., 1996). Furthermore COX-2 deficient mice have shown improvement following injury after N-methyl-D-aspartate administration (Iadecola and Alexander, 2001) whereas COX-2 over expression exacerbates brain injury (Dore et al., 2003; Iadecola and Gorelick, 2005). Interestingly, COX-2 mediates its toxic effect through Prostaglandin E_2 rather than reactive oxygen species even though COX-2 can generate both (Manabe et al., 2004). Inflammatory processes play a fundamental role in stroke, in both the etiology of ischemic cerebrovascular disease and the pathophysiology of cerebral ischemia. But they are however, also considered to be a triggering factor for stroke, clinically (Lindsberg and Grau, 2003).

Nimesulide (a preferential COX-2 inhibitor) was shown to be effective against transient (Candelario-Jalil et al., 2004) as well as permanent middle cerebral artery occlusion in rats (Candelario-Jalil et al., 2005). Etoricoxib (5-chloro-2-[6-methyl pyridin-3-yl]-3-[4-methylsulfonylphenyl] pyridine) is a highly selective, second generation COX-2 inhibitor. The IC_{50} selectivity ratio (COX1/COX2) for inhibition of COX-2 by Etoricoxib is 106 as compared to 7.3 for Nimesulide (Riendeau et al., 2001). Pharmacological action of Etoricoxib has not yet explored in transient middle cerebral artery occlusion (tMCAO) model.

The objective of this study was to evaluate the protective effect of Etoricoxib against transient focal cerebral ischemia reperfusion induced injury in rats. Parameters investigated included; neurobehavioral impairment, biochemical alterations and infarct volume.

2. Materials and methods

2.1. Animals

Male Wistar rats, weighing 270 to 300 g, were purchased from National Toxicology Centre, Pune, India. The rats were housed in a 12-

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doi:10.1016/j.ejphar.2011.05.030

h light/dark cycle at ambient temperature and had free access to water and food. All experimental procedures were approved by the Institutional Animal Ethics Committee of Poona College of Pharmacy and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2. Materials

Etoricoxib was obtained as gift sample from Panacea Biotech, India and 2, 3, 5-triphenyltetrazolium chloride (TTC) was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India.

2.3. Treatment schedule

Selected animals were randomly distributed among 5 groups viz vehicle (n = 25), SHAM (n = 6), ETX (1) (n = 15), ETX (3) (n = 10) and ETX (10) (n = 15). In the vehicle and SHAM group, 0.25% sodium carboxy methyl cellulose, in normal saline, was injected intraperitoneally (i.p.) immediately after tMCAO. Etoricoxib (1, 3 and 10 mg/kg i.p) was administered in three test groups designated as ETX (1), ETX (3) and ETX (10) respectively, immediately after tMCAO. The dose selection was based on the previous reports (Capone et al., 2005; Patrignani et al., 2003; Riendeau et al., 2001; Whiteside et al., 2004).

2.4. Transient middle cerebral artery occlusion (tMCAO)

Focal cerebral ischemia was induced by intraluminal suture method as reported previously (O'Neill and Clemens, 2001). In brief, the rats were anesthetized with ketamine (80 mg/kg i.p.) and xylazine (6 mg/kg i.p.). Rectal temperature was maintained at 37 °C with a heating pad for the duration of surgery and the immediate postoperative period until the animal recovered fully from anesthesia. A longitudinal incision of 1.5 cm was made in the midline of the ventral cervical skin. The left common carotid artery, left internal carotid artery, and left external carotid artery were exposed. The distal portion of external carotid artery was ligated and cut. A heparinized 3-0 Ethilon™ polyamide black monofilament (NW3328) of 40 mm length and tip rounded by heating near glowing ember (final tip diameter ~ 0.4 ± 0.02 mm) was inserted into the stump of left external carotid artery. To occlude the blood flow to the middle cerebral artery territory, the filament was advanced 19–21 mm from the bifurcation of common carotid artery into the left internal carotid artery until resistance was felt. The filament was held in place by tightening the suture around the external carotid artery and placing a microvascular clip around the artery. The wound was closed, and the animal was returned to its cage. To allow reperfusion, the filament was withdrawn after 120 min of middle cerebral artery occlusion, and then the external carotid artery was closed permanently by electro coagulation. The SHAM operations were performed in a similar manner except that the suture was withdrawn immediately after it was inserted. Successful middle cerebral artery occlusion was verified, after the animal recovered from anesthesia, using the neurological deficit test which correlates with infarct volume (Garcia et al., 1995).

The dynamic changes of the micro vessel occlusion in this model have been characterized. General physiological parameters such as non invasive blood pressure and electro cardio gram were recorded 1 h before, 30 min after and at 24 h of tMCAO with the help of 8 Channel recorder system of PowerLab (AD Instruments).

2.5. Neurological deficit scoring

Neurological deficit scores were recorded 1 h before and at 3 and 24 h after tMCAO. Neurological deficit was scored on an 18 point scale as described earlier (Garcia et al., 1995). The observational parameters were (1) spontaneous activity (0 to 3 points); (2) symmetry in the movement of four limbs (0 to 3 points); (3) forepaw out-stretching (0 to 3 points); (4) climbing (1 to 3 points); (5) body proprioception (1 to 3 points); and (6) response to vibrissae touch (1 to 3 points). The score given to each rat at the completion of evaluation at each time point is the summation of all six individual test scores. The minimum neurological score is 3 and the maximum is 18. Only the rats manifesting score of 9 to 11 at 3 h after tMCAO were included in the study.

2.6. Measurement of locomotor activity (ambulation) by actophotometer

The locomotor activity (ambulatory activity) was recorded using actophotometer (IMCORP, India). Animals were placed individually in the activity meter for 3 min for habituation. Thereafter, locomotor activity was recorded for a period of 5 min. Ambulatory activity was recorded and expressed in terms of total photo beam counts per 5 min (Bodhankar et al., 2007; Gaur et al., 2009).

2.7. Rotarod activity

All animals were evaluated for grip strength and balance using the rotarod. Each rat was given a prior training session before initiation of therapy to acclimatize them on a rotarod apparatus (Techno, Ambala, India). Animals were placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). Three separate trials were given to each rat at 5 min interval and cut off time (180 s) was maintained throughout the experiment. The average results were recorded as fall of time (Gaur and Kumar, 2010c).

2.8. Dissection and homogenization

After 24 h, animals were randomized into two groups. The first group of animals was used for biochemical and the second group for mitochondrial complex enzyme estimation after behavioral assessments. In the biochemical analysis, animals were euthanized by decapitation. Striatum was separated from each isolated brain. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate were centrifuged at 10,000 × g at 4 °C for 15 min. Aliquots of supernatants were separated and used for biochemical estimations.

2.9. Measurement of oxidative stress parameters

2.9.1. Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation (LPO) in striatum was performed according to the method of Wills. The amount of malondialdehyde (MDA), a measure of lipid peroxidation was measured by reaction with thiobarbituric acid at 532 nm using Jasco V-650 spectrophotometer (Oklahoma, USA). The values were calculated using molar extinction coefficient of chromophore ($1.56 \times 105\ M^{-1}\ cm^{-1}$) and expressed as percentage of vehicle treated group (Kumar et al., 2009; Wills, 1966).

2.9.2. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediame dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. Equal volumes of supernatant and Greiss reagent were mixed and incubated for 10 min at room temperature. The absorbance of each sample was determined at 540 nm at Jasco V-650 spectrophotometer (Oklahoma, USA). The concentration of nitrite in the supernatants was determined from a sodium nitrite standard curve and expressed as percentage of vehicle treated group (Green et al., 1982; Kumar et al., 2010b).