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Effect of Etoricoxib in the cerebral hypoperfusion induced on memory impairment and hippocampal damage in rats

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Abstract

Etoricoxib is one of the most often prescribed non-steroidal anti-inflammatory drugs. It selectively inhibits cyclooxygenase-2 (COX-2). COX II inhibitors recently had shown to have neuroprotective properties in animal models of acute neurologic injury. We assessed the effect of anti-inflammatory drug Etoricoxib, a selective COX II inhibitor on cerebral hypoperfusion injury. It has been reported that behavioral and histopathological abnormalities are occur by long-term cerebral hypoperfusion induced by permanent BCCA occlusion. Male Sprague–Dawley rats were randomly divided into a sham-operated group, a control group, and a model group with permanent BCCA occlusion. Etoricoxib 10 mg/kg was administered to model group. In the present study, as tested by open field paradigm and Morris' water maze, a propensity towards anxiety and disturbances of learning/memory were observed in animals subjected to hypoperfusion for 2 weeks.

Etoricoxib pretreatment (10 mg/kg/day) significantly reduced these hypoperfusion induced functional disturbances. Moreover, in histopathological evaluation of rat forebrain subjected to hypoperfusion there were reactive changes in the form of gliosis, astrocytosis and cellular edema. While Etoricoxib pretreatment showed the decreased severity in reactive changes. The results suggest that Etoricoxib may be useful in treatment of cerebral hypoperfusion injury and this effect may be due to possible anti-inflammatory activity. Further research is needed on COX II inhibitor's role in neuroinflammation.

Keywords: *Etoricoxib, Hypoperfusion, Neuroprotection, Neuroinflammation.*

Introduction

Bilateral common carotid artery (BCCA) occlusion-induced long-term cerebral hypoperfusion reduces blood flow from nearly 30–45% in the cortex to 20% in the hippocampus, resulting in a 20–30% and 15% decrease in glucose utilization, respectively [1, 2]. It has been frequently demonstrated that this chronic reduction in blood flow causes behavioral and cognitive abnormalities [3,4] and that it lasts to a significant degree for at least one month following permanent BCCA obstruction [5]. Non-steroidal anti-inflammatory agents (NSAIDs) have anti-inflammatory, analgesic, and antipyretic effects. They also block the enzyme cyclooxygenase (COX), which is involved in the production of prostaglandin precursors from arachidonic acid. NSAIDs also inhibit activation of neutrophils, which induce inflammation by releasing products COX-1 and COX-2, increasing prostaglandins, which may aggravate inflammation [6]. According to research, neurodegenerative diseases and neuroinflammation is responsible for the activation of glial cells, primarily microglia and astrocytes, and the generation of important inflammatory mediators as well as damaging free radicals are characteristics of inflammation in the brain. Etoricoxib is a selective inhibitor of cyclooxygenase-2 (COX-2), and it is one of the most prescribed non-steroidal anti-inflammatory drug. COX II inhibitors recently had shown to have neuroprotective properties in animal models of acute neurologic injury [7]. Etoricoxib has beneficial effects against transient middle cerebral artery occlusion model in rats. Maheshwari et al reported that the Etoricoxib may be considered as a potential candidate in the treatment of stroke, clinically [8]. Thus, the purpose of the current study was to further assess the impact of Etoricoxib on long-term hypoperfusion (permanent BCCA occlusion for two weeks). Parameters investigated included neurobehavioral impairment, biochemical alterations and histopathological investigation.

Materials and Methods

Animals

Male wistar rat (180-225g) was employed in the research. They were kept in conventional climatic settings, with a temperature of 25 +/- 2°C and a relative humidity of 45–55% (12 h light 12 h dark cycle).

The animals had free access to food and water ad libitum. The food was withdrawn 18 h prior to surgical procedure. The protocol was accepted by the Institutional Animal Ethical Committee (approval number: CPCSEA/IAEC/PC-04/09-2K7). Every experiment was run from 12:00 to 16:00.

Chemicals and Drugs

The Etoricoxib injection was bought from a local pharmacy. Additional chemicals were bought from an approved vendor and were of the quality of analytical grade.

Experimental Procedure

Surgical Procedure

The surgical approach used to induce cerebral hypoperfusion was modified from an earlier Iwasaki et al. published method [9]. In brief, a midline skin incision in the neck was performed under anesthesia with ketamine (80 mg/kg i.p.). The vagosympathetic nerve was properly identified and isolated from the common carotid arteries. The hypoperfusion was achieved by permanent BCCA. A heating lamp helped to keep the body temperature at 37 °C during that time. The carotid arteries were severed in between and doubly ligated using 3-0 silk sutures for long-term hypoperfusion experiments. After suturing the skin, the animals were put back in their original cage [10].

Final Steady state experiment

The animals were split up into three groups of six each for hypoperfusion studies and experienced permanent BCCA blockage. Sixty minutes before BCCA occlusion, Etoricoxib 10 mg/kg was given to the third group. Then, Etoricoxib was administered until the fifteenth day following surgery. All the animals were put through behavioral testing in the Morris water maze and open field paradigm on day 15, 60 minutes after their last Etoricoxib dosage. Following cervical dislocation, the animals were killed, and brain tissues were taken for histological examination [10].

Behavioral testing

Open field test

An open field paradigm was used to assess locomotor activity [11]. The plywood-constructed open field had high walls and a 96 x 96 centimeter floor. The entire equipment was painted black, with the exception of the 16 squares of partitioned floor that were 6 mm thick in white. Every animal was put in a corner of the device, and during the course of the five minutes, its ambulations (the number of squares it crossed), total immobility (measured in seconds), number of rearings, groomings, and fecal pellets were all recorded.

Morris Water Maze

Spatial learning and memory were assessed in a water maze on day 15 following surgery [10, 12]. Within the maze was a black circular pool (width 2.14 m, height 80 cm) that was 44 cm deep and contained 25oC water. The rats were habituated on the fourteenth day, spending one minute in a water labyrinth without a platform. On the fifteenth day, a circular platform with a diameter of 9 cm was concealed in the center of one of the quadrants, 2 cm below the water's surface. Throughout the training sessions, the platform stayed in the same location. A random sequence of four starting poles was generated around the pool's circumference at the start of each session.

This order was followed by every animal during that session. Every rat was started in the water with its back to the wall, and it had ninety seconds to locate the concealed platform. The animal was permitted to rest on the platform for 20 seconds. The time it took to get to the platform was noted. The rat was hoisted out and left on the secret platform for 20 seconds if it could not find it. Every one of the four start places underwent the same process.
On the initial testing day, there were two sessions of four trials each, planned four hours apart, and one session of four trials the following day (reference memory technique). The platform was then taken down, and four hours later, a probe trial was held without a platform. Every rat was dropped into the pool at the same randomly chosen beginning point, and its swimming route was tracked. The amount of time spent in the pool's quadrant with the platform at first was measured (working memory process).

Histopathology

Rats were sacrificed by beheading and having their brains removed when the behavioral testing was completed. After that, the brains were placed in 10% formalin. Hematoxylin and eosin was used to make frontal slices, each of which was 0.05 mm thick. Under light microscopy, stained sections were assessed qualitatively by an examiner who was blind to the experimental circumstances [10].

Statistical Analysis

Statistical analysis of data was performed by applying one way analysis of variance (ANOVA) followed by Tukey test for biochemical parameters and Mann–Whitney U test for behavioral observations. A p value of b0.05 was considered statistically significant.

Results

Behavioral Observation

Animals with permanent BCCA blockage (hypoperfusion) displayed significant changes in locomotor activity in open field behavior tests (Table 1). Animals that were hypoperfused showed fewer ambulations and rearings as their immobility duration increased. Even though there were fewer groomings than in the control group,

the difference was not statistically significant. Treatment with etoricoxib stopped these changes, which led to a decrease in the amount of time spent immobile and an increase in the frequency of ambulations and rearings.

Behavioral Observation

The outcomes of Morris' water tests are compiled in Figs. 1, 2, and 3. During the habituation testing, every animal swam normally. During the escape trial, all of the rats found the hidden platform, although it took the hypoperfused rats longer than the sham-operated controls. This delay in escape latencies was avoided with etoricoxib therapy. In contrast to the first session, all of these modifications peaked in the second and third sessions. Hypoperfused rats spent less time in the quadrant of the old platform position during the probing experiment than did sham-operated controls and hypoperfused animals treated with etoricoxib, according to an analysis of swimming performance. Once more, the groups were very different. These metrics showed no effect from etoricoxib alone.

Table 1. Effect of Etoricoxib extract on open field behavioral parameters in hypoperfused rats

Groups	Ambulations (number)	Immobility (Second.)	Rearings (number)	Groomings (number)	Fecal pellets (number)
Sham operated	54.83±3.12	31.68±1.7	24.5±2.14	4.16±1.07	2.16±0.3
Hypoperfused	26.33±2.96 ***	45.1±1.86 ***	15.16±2.08*	9.00±1.15*	2.66±0.42
Etoricoxib 10 mg/kg Treated	51.16±3.1###	31.5±1.45#	20.83±2.1ns	8.5±1.25#	2.33±0.33ns

Results are expressed as mean ± SEM. (n = 6). Control and treatment groups are compared with hypoperfusion group. Data was analysed by one way analysis of variance (ANOVA) followed by Tukey test. *,# P<0.05, **,##P<0.01, ***,###P<0.001. *- Ischemia reperfused mice compared against sham operated rats, #- Etoricoxib pretreated mice compared against ischemia reperfused rats.

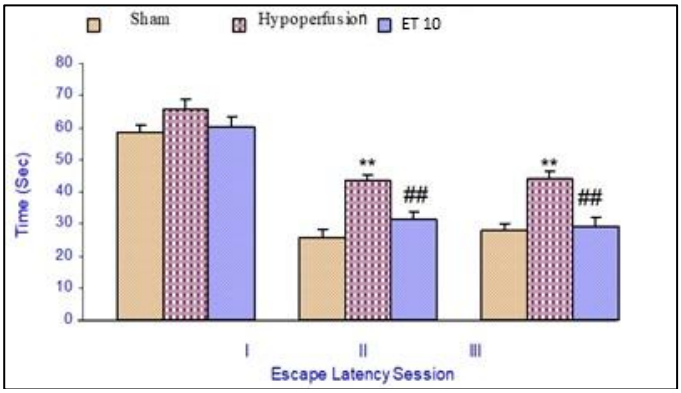

Figure 1. Effect of Etoricoxib extract on the Escape latency (second) using morris water maze in permenant BCCA occluded rats

Results are expressed as mean ± SEM. (n = 6). Control and treatment groups are compared with hypoperfusion group. Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *,# P<0.05, **,##P<0.01, ***,###P<0.001. *- Ischemia reperfused mice compared against sham operated rats, #- Etoricoxib pretreated mice compared against ischemia reperfused rats.

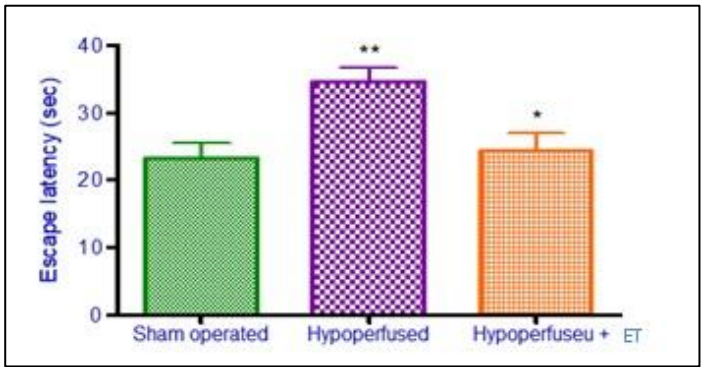

Figure 2. Effect of Etoricoxib extract on the Escape latency in new platform trial (second) using morris water maze in permenant BCCA occluded rats

Results are expressed as mean ± SEM. (n = 6). Control and treatment groups are compared with hypoperfusion group. Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05. **P<0.01.

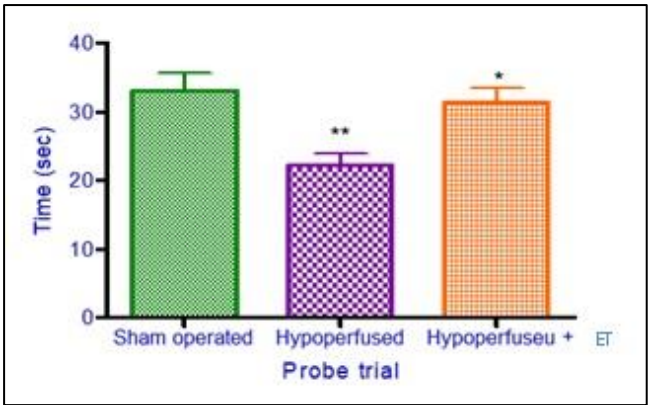

Figure 3. Effect of Etoricoxib extract on probe trial (second) using morris water maze in permenant BCCA occluded rats

Results are expressed as mean ± SEM. (n = 6). Control and treatment groups are compared with hypoperfusion group. Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05. **P<0.01.

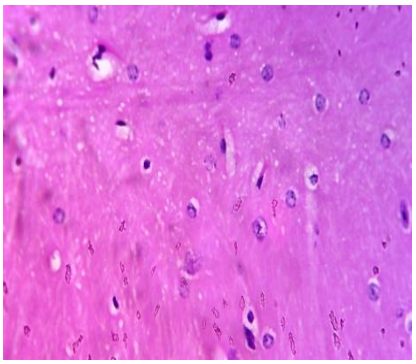

Figure 4A. Histopathological alterations in the forebrain of a control rat that was sham-operated. Take note of the brain's typical architecture.

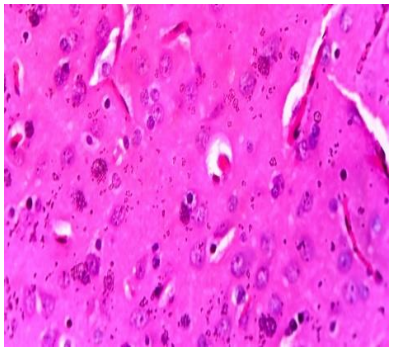

Figure 4B. Histological alterations in the rat forebrain after hypoperfusion. Take note of reactive alterations such as cellular edema, gliosis, and astrocytosis.

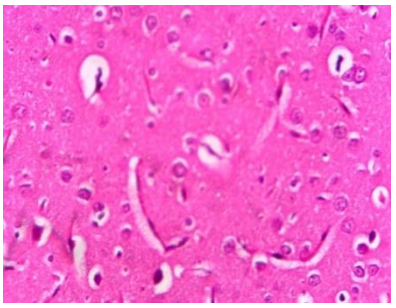

Figure 4C. Etoricoxib's (10 mg/kg, po for 15 days) impact on the histological alterations in the rat forebrain after hypoperfusion. Observe how the response changes have lessened in intensity.

Figure 4. Histopathological changes in rat forebrain.

Histopathological Observations

The normal histological architecture of a rat brain in an animal model of surgery is depicted in Figure 4A. After two weeks of hypoperfusion, the brains of the animals showed abnormalities in brain histology, including cellular edema, perivascular infiltration, increased glial cell count, and the presence of macrophages (Figure 4B). As evidenced by less inflammatory changes, a milder lymphocytic infiltration, and less glial cell proliferation, Etoricoxib attenuated these hypoperfusion-induced alterations (Figure 4C).

Discussion

Rats with permanent BCCA occlusion have been employed as animal models for dementia, white matter lesions, neurodegenerative diseases, and cerebrovascular insufficiency syndromes [13–15]. According to established principles, the current study's research on open field behavior revealed that long-term hypoperfused animals were more likely to experience anxiety. This long-term worry caused by hypoperfusion has been greatly avoided by Etoricoxib.

According to reports, a chronic decrease in blood flow brought on by BCCA obstruction might lead to increasing dysfunction and cognitive deficiencies [1, 3]. Due to a flawed learning process, long-term hypoperfused animals regularly required more time to locate the submerged platform. When paired with the probing trial results, this illustrates how long-term hypoperfused rats' working and reference memory are disrupted. In long-term hypoperfused rats, Etoricoxib dramatically reduced these changes, suggesting a beneficial role for Etoricoxib in learning and memory.

The rat forebrain that had been hypoperfused showed reactive alterations in the form of gliosis, astrocytosis, and cellular edema in the histological examination. On the other hand, Etoricoxib pretreatment demonstrated less severe response alterations [8].

Etoricoxib is a selective cox-II inhibitor, most commonly used NSAIDs to reduce the pain, inflammation .The main mechanism of action of etoricoxib is to inhibit the cyclo-oxygenase enzyme which is responsible for neuroinflammation in brain .Our findings identified that these drug to exert not only their anti-inflammatory, analgesic, antipyretic activities but also neuroprotective activities against neurodegeneration.

Conclusion

Etoricoxib 10 mg/kg showed neuroprotective effect by significant prevention of long term hypoperfusion induced neuronal alterations. Etoricoxib will be effective in cerebrovascular insufficiency, neurodegenerative disorders, and dementia.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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