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Perindopril Possesses a Protective Impact on Neuronal Function by Lowering Inflammation and Oxidative Stress in Adult Male Rats During Ischemia/Reperfusion

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Abstract: **Background:** Cerebral ischemia/reperfusion (CI/R) is a separate feature of ischemic stroke that occurs when blood perfusion is restored following a period of ischemia. Despite the beneficial effects of reperfusion, it can cause even more harmful outcomes than persistent ischemia. **Objectives:** This study was conducted to assess perindopril's neuroprotective ability in (CI/R) and to conceptualize its molecular underpinnings. **Materials and Methods:** The 28 Sprague-Dawley male rats weighing 200-300 g were randomly divided into four groups, each with seven rats: Sham (undergo general anesthesia without bilateral common carotid artery occlusion (BCCO)), Control (30 min. of BCCO followed by reperfusion 60 minutes), Vehicle (7 days of distilled water then as control group), and Perindopril-treated group (7 days of Perindopril-treated then as control group). After reperfusion, the brain's tissues were extracted in order to evaluate the levels of biochemical, and total antioxidant capacity in addition to the extent of the infarct and histological investigation. **Results:** Cerebral infarct size, as well as levels of inflammatory marker were significantly higher in the control and vehicle groups compared to the sham groups, although overall anti-oxidant capability was significantly lower. Perindopril therapy increased IL-10 and overall antioxidant capacity while decreasing IL-6, TNF-α, NF-kB p65, and ICAM-1 significantly. Histopathological, both control and vehicle rats had severe ischemia damage, which was significantly alleviated by perindopril therapy. **Conclusions:** The perindopril neuroprotective effect in rats exposed to cerebral ischemia/reperfusion injury may be attributed to the anti-inflammatory and anti-oxidative effect of perindopril.

Key Words: perindopril; cerebral ischemia-reperfusion; inflammation; total anti-oxidant capacity

I. INTRODUCTION

Stroke is a major cause of death and disability worldwide. The stroke mean age is within the sixth and the seventh decade, ranging from 59-71, with median of 65 years. Stroke incidence more than doubles in each consecutive decade for persons over 55 years old [1]. Ischemic stroke is the most described type of stroke (about 87%), then intracerebral hemorrhage (10%), and subarachnoid hemorrhage (3%).

Ischemic stroke resulted from critical drop of cerebral blood flow continuing beyond a critical duration. Ischemia could be due to arterial thrombosis, embolism, systemic hypoperfusion, or venous thrombosis. The brain is highly susceptible to ischemia due to its very high oxygen demands. Complete disruption of cerebral blood flow for just 5 minutes induces the death of susceptible neurons. The CI/RI denotes to a period of reduced cerebral perfusion, followed by recovery of brain blood supply. It is well-known that reperfusion after brain ischemia leads to secondary injury associated with structural and functional damage causing paradoxical dysfunction and necrosis of tissue.

The stroke acute stage starts within minutes and might persists for days or weeks following ischemia [2]. Without satisfactory blood supply and oxygen, brain cells lose their capability to create energy, mainly ATP by mitochondria. Cells in the ischemic region shift to anaerobic glycolysis, that leads to a lesser ATP production and lactic acids release causing cell acidosis. Without adequate energy, the ATP-dependent Na^+/K^+ pumps drop, leading the cell membrane to depolarize; causing a larger intracellular influx of Na^+, Ca^{+2}, and H+ ions with K^+ efflux that lead to an in-

crease in the cellular osmolality, water retention and cellular swelling. Intracellular Ca^{+2} levels become very high and causes the excitatory amino acid neurotransmitter glutamate to be released, which stimulates AMPA receptors and Ca^{+2}-permeable NMDA receptors, resulting in even more Ca^{+2} intracellular influxes. The excessive Ca^{+2} entry cause activation of intracellular proteases leading to cell autolysis. The generation of the free radicals triggers the gene expression of inflammatory cytokines and chemokines [3]. As the cell membrane is destructed by phospholipases, it come to be more permeable causing further edema. The mitochondrial break down releases apoptotic factors and toxins into the cell leads to apoptosis. When the cells necrosis ensues, it liberates great amounts of glutamate and toxic compounds into the neighboring cells causing more injury (Excitotoxicity) [4].

While reperfusion following brain ischemia can restore blood flow and rescues reversibly injured tissue, it can paradoxically trigger more cell damage and necrosis. The mechanisms leading to neuronal damage in CI/RI are complicated and multifactorial. They include infiltration of leukocytes, generation of inflammatory cytokines and chemokines, mitochondrial injury, oxidative stress, complement and platelet stimulation, and disruption of the blood-brain barrier leading to neuronal.

Perindopril is a prodrug that inhibits angiotensin-converting enzyme (ACE). It is converted to perindoprilat, its active form, via hepatic first pass effect (62%), and systemic hydrolysis (38%) [5].

II. MATERIALS AND METHODS

The Kufa College of Medicine's Department of Pharmacology was where the study was conducted. 200-300g weight of male 28 Sprague-Dawley rats were purchase from the College of Science/Kufa university. The animals were maintained in a well-ventilated animal housing with a 12 hour/12-hour light/dark cycle and were fed a conventional animal food along with plenty of water. The Laboratory Animals Guide to Care was followed during the inquiry, and the experiment received approval from the Al-Kufa University Animal Care and Research Committee.

ANIMALS GROUPS

In group 1 (the sham group), general anesthesia was administered to the rats, but BCCAO was ignored. The control group's rats in group 2 got BCCAO for 30 minutes just before getting an hour of reperfusion. Group 3 (Vehicle group): The rats received the same dose of DW orally by oral gavage for 7 days prior to the procedure as did the Control group. Rats in Group 4 (the perindopril-treated group) underwent gastric gavage administration of the medication at a dose of 4 mg/kg/day for 7 days prior to receiving the same procedure as the control group. Procedure protocol: The rats are given general anesthesia through intraperitoneal injections of xylazine (10 mg/kg) and ketamine (100 mg/kg). Through a median neck incision, both common carotid arteries were seen, and they were then blocked for 30 minutes to cause ischemia. Reperfusion was allowed a full hour after the clamps were released. Rats were put to death, and their brains were removed, kept as cold as possible, and coronally sectioned for ELISA, IHC, TTC stain, and histopathological investigation.

Tissue Samples Preparation: Brain tissue was homogenized and the supernatants' levels of IL-6, IL-10, ICAM-1, TNF-, and T-AOC were measured and computed. [6]

Histopathology: The brain samples were processed and stained with hematoxylin and eosin. Double-blinded pathologists assigned the following scores to the changes [7], [8]; Zero (Normal): No harm. 1 (modest): dark-colored or Eosinophil cells or small interstitial edema. 2 (Moderate): at least two minor bleedings. Acute necrosis, grade 3 (severe).

Infarct Size: Using a digital camera and image analysis software (the image J system), the infarct volume was calculated. Using Swanson's approach, the infarct area was assessed. The total volumes of both white area (infarction area) and red area (valid area) were measured, and the infarct percentage (I) was calculated as:

$$I = (\text{White Area})/(\text{Total Area}) \times 100.$$

STATISTICAL ANALYSIS

SPSS version 26 was used to analyze the data. Parametric data were evaluated using the ANOVA test, followed by the LSD post hoc test, after the Shapiro-Wilk test was used to check for normality. Kruskal-Wallis and post hoc tests are used in a non-parametric test. P-values under 0.05 were regarded as statistically significant in all categories.

III. RESULTS

PERINDOPRIL REDUCES LEVELS OF CEREBRAL INFLAMMATORY AND ROS MARKERS

The current investigation found that the control and vehicle-related to sham groups had substantially higher levels of cerebral IL-6, IL-10, TNF-α, and ICAM-1 ($p < 0.05$). The pretreatment with perindopril significantly lowered ($p<0.05$) the increments of IL-6, TNF-α, and ICAM-1 while significantly increasing ($p<0.05$) the increments of IL-10 (Figures 1 and 2). Control and vehicle rats had significantly less cerebral

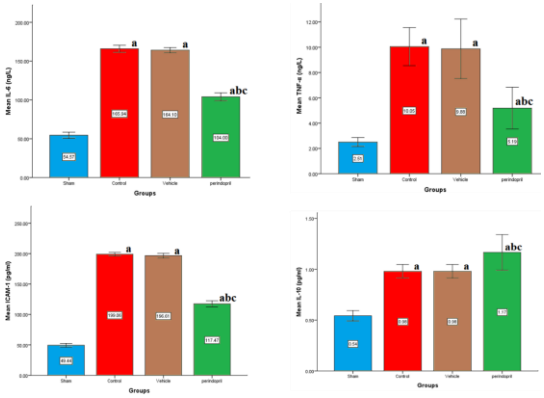

FIGURE 1: The Difference in IL-6 (ng/L), TNF-α (ng/L), ICAM-1 (pg/ml), and IL-10 (pg/ml) Levels Among Groups

T-AOC than sham rats ($p < 0.05$) in comparison. These levels were considerably increased by the administration of perindopril ($p<0.05$) as compared to the control and vehicle groups (Figure 2).

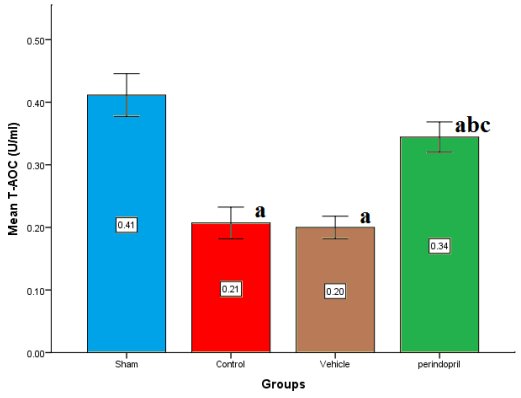

FIGURE 2: The Difference in T-AOC (U/ml) Levels Among Groups

PERINDOPRIL REDUCES LEVELS OF CEREBRAL NF-κB P65

In our investigation, NF-B p65 expression was considerably greater in the control and vehicle groups compared to the sham group ($p < 0.05$). The pretreatment with perindopril significantly reduced this obvious rise ($p < 0.05$) (Figures 3 and 4).

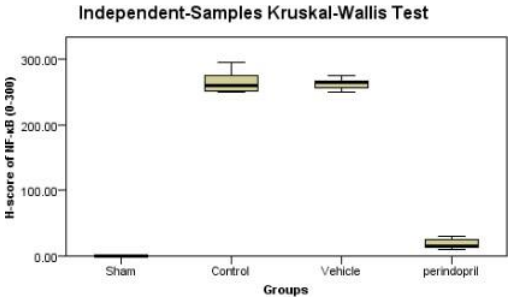

FIGURE 3: The Difference in NF-κB Expression Levels Among Groups

Perindopril reduces cerebral histopathological score: All of the sham animals underwent histopathological evaluation and seemed normal. Significant ischemia damage was evident in the control and vehicle groups ($p < 0.05$). These alterations were significantly ameliorated in perindopril-pretreated rats ($p < 0.05$) (Figures 5 and 6).

Perindopril reduces cerebral infarct size: According to the current study, the percentage of cerebral infarcts was significantly higher in the control and vehicle groups as compared to the sham groups ($p < 0.05$). In the group receiving perindopril, this proportion was considerably reduced ($p < 0.05$) (Figures 7 and 8).

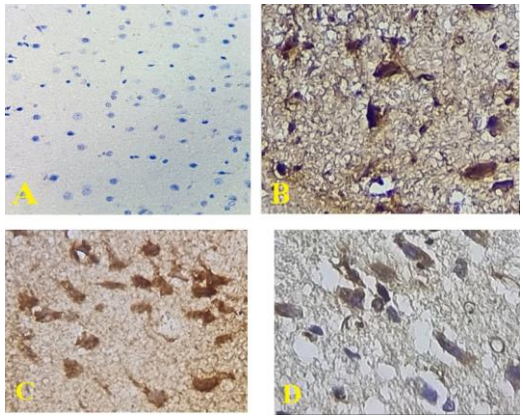

FIGURE 4: Rat Brain NF-κB Nuclear Expression as Shown by IHC. Sham Group's Negative Expression (X200) is in (A). Control Group (B) and Vehicle Group (C) with Strong Nuclear Expression (X400). Weak Expression (400) in the Perindopril-Treated Group

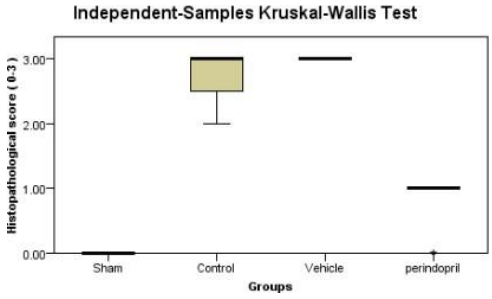

FIGURE 5: Histopathological Ischemia Alterations in Each of the Four Groups are Represented by Medians in a Kruskal Wallis Box Plot. Perindopril Treatment Compared to Control and Vehicle Groups and Sham Treatment Compared to Control and Vehicle Groups Showed a Significant Difference ($p<0.05$)

IV. DISCUSSION

The proinflammatory cytokines and reactive oxygen species are expressed in the sham group brain at low levels and are crucial for carrying out regular physiological processes. Strong inflammatory reactions brought on by the CI/RI might result in significant releases of these inflammatory mediators, which can worsen the original ischemia's effects. A significant problem is how a fresh treatment approach can disrupt these networks.

Effects of perindopril on cerebral IL-6, IL-10, TNF-α, ICAM-1, T-AOC, and NF-κB p65: In the current study, IL-6, TNF-α, ICAM-1, and NF-κB were markedly decreased ($p<0.05$) whereas IL-10 and T-TAC were markedly increased ($p<0.05$) by perindopril pretreatment in respect to the control and vehicle groups. These results denote that perindopril has anti-inflammatory and anti-oxidative properties which may ameliorate the brain injury caused by CI/RI.

Kamel et al. (2019) [9] studied perindopril effect on hep-

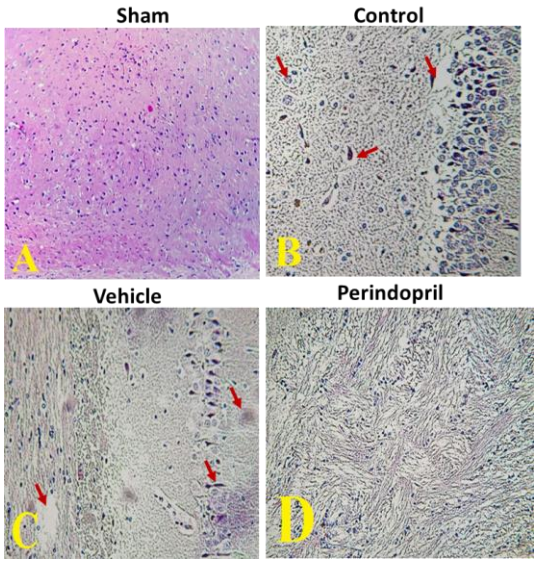

FIGURE 6: Photomicrograph of a Section for (A) the Sham Group Shows the Normal Architecture. (B) Control Group and (C) Vehicle Group Showed Edema, Hemorrhage, and Necrosis. (D) Perindopril-Treated Group: Mild Interstitial Edema

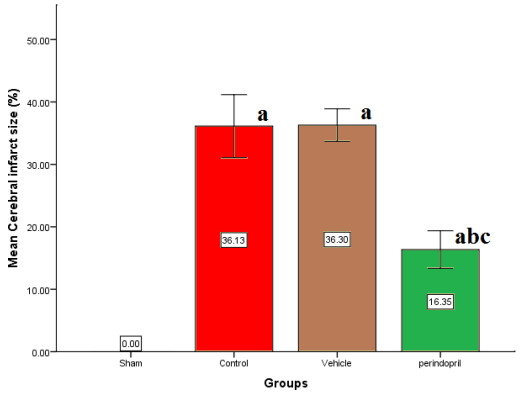

FIGURE 7: The Mean of Cerebral Infarct Size Percentage of the Four Groups

atic ischemia reperfusion injury in adult male rats and found that perindopril pretreatment remarkably decreased ($p<0.05$) both serum TNF-α levels and hepatic NF-κB p65 mRNA gene expression, meanwhile serum levels of IL-10 was considerably elevated in comparison to ischemia-reperfusion group.

Additionally, perindopril treatment resulted in marked amelioration of hepatic oxidative stress markers like sodium dismutase and malondialdehyde related to ischemia-reperfusion group. Mashhoody et al. (2014) [10] found that oral perindopril pretreatment at doses of 1, 2, and 4mg/kg/day for 4 weeks in adult male rats resulted in significant increment of reduced glutathione, while malondialdehyde level didn't altered in the hippocampus area, indicating

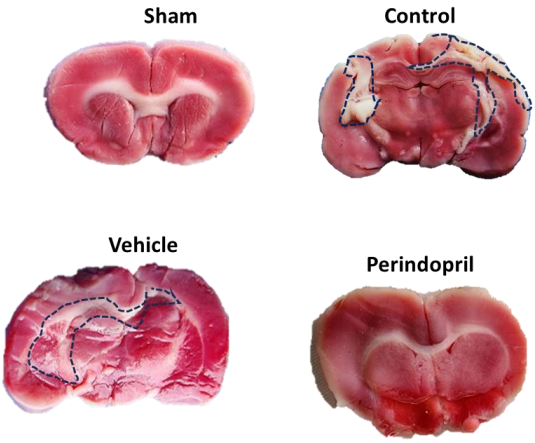

FIGURE 8: Photographs of Coronal Brain Slices Stained with TTC

that perindopril increases the antioxidant defense.

For best of our knowledge, there are no studies on perindopril effects on cerebral IL-6, IL-10, TNF-α, ICAM-1, T-AOC, and NF-κB levels of rats exposed to CI/RI. Perindopril-treated group displayed a significant amelioration in the ischemic changes indicating that perindopril has beneficial effects in CI/RI. The ischemic changes in perindopril group were mild including mild interstitial edema, few dark eosinophilic neurons, few hemorrhage, and healthier neurons.

Coskun et al. (2010) [11] studied perindopril effect on permanent cerebral ischemia in rats and found that histopathological damage was significantly less intense in perindopril pretreatment group compared to ischemic group.

As far as we know, there are no studies on perindopril effects on cerebral histopathology in rats exposed to transient cerebral ischemia. The current study showed that there was a marked reduction ($p<0.05$) in the cerebral infarct size percentage in perindopril-treated group in relation to control groups the referring to the neuroprotective effect of perindopril in CI/RI, see [12], [13].

V. CONCLUSION

According to the results of the current investigation, perindopril significantly reduces the size of cerebral infarcts and lessens histological damage in male rats that have undergone CI/RI. The anti-inflammatory and anti-oxidative characteristics of perindopril are likely the source of its neuroprotective benefits.

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This research paper received no external funding.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this paper. They have all read and approved the final version.

CONSENT

Informed consent was obtained from all participates in the study as needed.

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