Lacidipine, thiamine pyrophosphate and their combination on the ocular ischemic syndrome induced by bilateral common carotid artery ligation

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Abstract

- **AIM:** To investigate the effect of lacidipine, thiamine pyrophosphate (TPP) and the combination of lacidipine and TPP against oxidative and inflammatory eye damage induced by bilateral common carotid artery ligation in rats.
- **METHODS:** Male albino Wistar rats were categorized as those who underwent sham surgery (SG), right and left common carotid cross-clamping and unclamping procedure (CCU), lacidipine+CCU (LCCU), TPP+CCU (TCCU), and combination of lacidipine and TPP (LTC)+CCU (LTCCU). One hour before anesthesia, the LCCU (n=6) received lacidipine (4 mg/kg, orally) and the TCCU (n=6) received TPP (20 mg/kg, intraperitoneally). The SG (n=6) and CCU (n=6) received the same volume of distilled water from the same route. After anesthesia (60 mg/kg ketamine, intraperitoneally), the necks of the rats were opened in the midline. Ischemia was created for 10min by placing clips on the right and left common carotid arteries. Rats in the SG only underwent subcutaneous incision. After 10min, the clips were removed and reperfusion was achieved for six days. Then, the animals were euthanized (120 mg/kg ketamine, intraperitoneally) and the levels of oxidant, antioxidant and proinflammatory cytokines in the eye tissues were determined. The retinal tissue of the eye was also examined histopathologically.
- **RESULTS:** Lacidipine, TPP, and LTC significantly prevent the increase in malondialdehyde, tumor necrosis factor-alpha, interleukin-1β (IL-1β), and IL-6 levels, decrease in total glutathione levels, superoxide dismutase and catalase activities and histopathological retinal damage in eye tissue induced by bilateral common carotid artery ligation in rats. The impact of these drugs on protection is determined to be LTC>lacidipine>TPP.
- **CONCLUSION:** As a result of the study, it is concluded that LTC may be more effective than lacidipine and TPP alone in treating ocular ischemic syndrome.
- **KEYWORDS:** ocular ischemic syndrome; lacidipine; thiamine pyrophosphate; oxidative stress

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INTRODUCTION

Diseases that cause total or subtotal occlusion of the carotid artery play a significant role in the development of ischemia in the eye. Symptoms of visual impairment accompanying subtotal or total occlusion of the carotid artery are referred to as ocular ischemic syndrome (OIS)[1]. The incidence of OIS is especially high in patients with insufficient collateral circulation between the right and left internal carotid arteries or between the internal and external carotid arteries[1]. The etiology of OIS may be attributed to atherosclerosis, dissecting aneurysms of carotid vessels, Takayasu arteritis, and Behçet’s disease[2]. A retinal hypoperfusion injury is a pathophysiological basis for OIS, which often results in severe vision loss[3]. An occlusion of both carotid arteries in animals causes oxidative stress in the retina. There has
also been a focus on the fact that oxidant levels increase, and antioxidative levels decrease in the retina\(^4\). Ischemia-reperfusion (I/R)-induced retinal cell damage is attributed to several mechanisms, including energy deficiency, an increase in intracellular calcium, the generation of free radicals, and an increase in proinflammatory cytokines\(^3\).

A voltage-operated L-type calcium channel blocker, vasoselective and antihypertensive, lacidipine was examined in this study regarding its effects on retinal I/R damage\(^6\-\(^7\). Lacidipine has been reported to have a protective effect on organs and tissues by inhibiting lipid peroxidation (LPO) reactions and glutathione depletion\(^7\). Furthermore, lacidipine has been experimentally demonstrated to have anti-inflammatory properties. The anti-inflammatory effect of lacidipine is attributed to its inhibition of proinflammatory cytokines\(^8\).

Thiamine pyrophosphate (TPP) was another drug we investigated for its effect on retinal I/R damage. It has been stated in the literature that TPP protects ovarian tissue by inhibiting the increase in oxidant and proinflammatory parameters\(^10\). It appears that lacidipine and TPP may be effective in the treatment of retinal I/R damage, based on the information. Also, the combination of lacidipine and TPP (LTC) may be more effective in treating retinal I/R damage than either agent alone. It was hence the purpose of this study to investigate biochemically the effects of lacidipine, TPP, and LTC against oxidative and inflammatory eye damage induced by bilateral common carotid artery ligation in rats, as well as to examine retinal tissue histopathologically.

**MATERIALS AND METHODS**

**Ethical Approval** Experiments were carried out in the laboratories of the Experimental Animal Application and Research Centre of Erzincan Binali Yıldırım University. The experiment was performed after approval from the local Animal Experiments Ethics Committee of Erzincan Binali Yıldırım University (Date: 30/03/2023, Decision No: 03/06 Number: E-85748827-050.06.04-252336).

**Animals** Thirty male Albino Wistar rats, as defined in the experimental design, were provided by the Experimental Animals Application and Research Centre of Erzincan Binali Yıldırım University. The experimental animals were housed and fed for 1 wk at a temperature of (22°C±2°C) and a 12-hour light/dark regime to facilitate their adaptation to the environment.

**Chemical Substances** Ketamine was procured from Pfizer Drugs Ltd. Sti (Turkey), TPP was procured from BioPharma (Russia), lacidipine was procured from Glaxo Smith Kline Drugs (Turkey) and sevoflurane liquid 100% was procured from AbbVie (Turkey).

**Animal Groups** The experimental animals were created (6 rats per group) as those who underwent sham surgery (SG), right and left common carotid cross-clamping and unclamping (CCU), lacidipine+CCU (LCCU), TPP+CCU (TCCU), and LTC+CCU (LTCCU).

**Surgical and Pharmacological Procedures** Surgery was performed under sterile conditions in the laboratory. Before anesthesia 1h, lacidipine (4 mg/kg) was administered orally to the LCCU and LTCCU. Lacidipine has been previously used at these doses in experimental rat studies\(^11\). Mennini et al\(^12\) also demonstrated that lacidipine reached the cerebral cortex 1h after oral administration in rats. TPP (20 mg/kg) was applied intraperitoneally (ip) in the TCCU and LTCCU. TPP at these doses has been previously used in a brain I/R model in rats\(^13\). In addition, it has been demonstrated that TPP administered 1h before the cerebral hypoxia model procedure in rats crosses the blood brain barrier and reaches the brain and has a healing effect\(^14\). With the same method, the same amount of distilled water was applied to the SG and CCU groups. This treatment procedure continued for 6d, 1 time per day. Ketamine hydrochloride (60 mg/kg, ip) was used for general anesthesia. Sevoflurane 3% was used at appropriate intervals to maintain anaesthesia. During this period, all rats were immobilized on an operating table in the supine position and their necks were shaved midline. Following disinfection, an incision was made in the midline. A superficial microdissection was followed by deep microdissection of the right and left common carotid arteries. During this procedure, the trachea was visualized, the paratracheal muscles were dissected, the common carotid artery was reached, and a clip was placed on the artery. For 10min, the clips were kept closed to create ischemia. The rats in SG received a subcutaneous incision but no clamping. The ischemia period was followed by the removal of clips, suturing of the incisions, and reperfusion for six days. Then, the animals were euthanized with ketamine (120 mg/kg, ip) and malondialdehyde (MDA), total glutathione (tGSH), superoxide dismutase (SOD), catalase (CAT), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and IL-6 were measured in removed eye tissues. The retinal tissue of the eye was also examined histopathologically.

**Biochemical Analyses**

**MDA, tGSH, SOD, CAT, and protein analyses** After washing with physiological saline, tissue samples were crushed by adding liquid nitrogen and homogenized. Supernatants were used for MDA, tGSH, SOD, and protein analysis. The determination of MDA (nmol/mg protein), tGSH (nmol/mg protein), and SOD (U/mg protein) enzyme-linked immunosorbent assay in eye tissues was performed according to the instructions provided with each assay kit (Cayman Chemical Company, product No. 10009055, 703002,
Histopathological Examination All eye tissue samples were kept in 10% formaldehyde solution for 72h for light microscopy evaluation. Later, the samples were taken into cassettes and kept under running water for 24h. It was then treated with graded alcohol (60%-100%) for dehydration. Eye tissues were made transparent using xylol and then placed in paraffin. Sections (4-5 μm) were obtained from paraffin blocks and stained with hematoxylin-eosin. An examination was conducted of the GCL, IPL, INL, OPL, and PRL and all layers were clearly defined. The nuclei of the INL and ONL were stained with a basophilic stain. A limited number of blood capillaries were observed in the GCL and the IPL.

In the retinal tissue of the CCU group, severe degenerative changes were identified with a damage severity grade of 3. There were significant reductions in ganglion cells in some samples, resulting from a disordered distribution of ganglion cells. Several changes in retinal blood vessel walls were observed, including thickening of the walls, dilatation, and congestion. There was intense edema in the GCL, as well as irregular groupings of nuclei in the INL and ONL. Numerous void areas observed in INL (Figure 1B, Table 2). In this group, the PRL had a foamy appearance and several areas of vacuolization were present. Observations indicated that there was significant degeneration and separation of the OPL, as well as irregular groupings of nuclei in the INL and ONL.

Statistical Analysis Statistical analyses were performed using IBM SPSS Statistics 22 Software. First, the Kolmogorov-Smirnov test was applied to the numerical data. For normally distributed parameters, one-way ANOVA was preferred in the analysis. The selection of post-hoc tests was made according to Levene’s test (Tukey HSD or Games-Howell) and data were presented as mean value±standard error. Kruskal-Wallis test followed by Dunn’s test was used for the analysis of non-normally distributed data and histopathological grading data. Data set was expressed as a median (maximum-minimum). Statistical significance was determined to be 0.05.

RESULTS
Biochemical Findings
MDA, tGSH, SOD, and CAT analysis results MDA levels in the CCU group were higher than those in healthy rats (P<0.001). TNF-α, IL-1β, and IL-6 levels were found to be higher in the CCU group than in sham operation-applied rats (P<0.001). In the SG group (Table 1).

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in the capillaries, and polymorphonuclear cells near the capillaries. A slight segmental fragmentation was observed in the PRL. As compared to the healthy group, the INL nucleus density was relatively decreased. Cell separation and moderate deterioration of connections were observed in the OPL. An overall image of the retina revealed mild edema (Figure 1D, Table 2).

The retinal tissues of the LTCCU group, with grade 0 damage severity, appeared to be similar to those of the healthy group, and the retinal layers appeared normal. The retinal blood capillaries had a normal appearance, and no polymorphonuclear cells were found. In both the inner and ONL, the nuclei of the cells were more organized, ordered, and of a higher density than those of the damaged groups. Damaged groups also showed a reduced separation of the OPL and exhibited a similar appearance to healthy groups. PRL was identical to that of the healthy group (Figure 1E, Table 2).

**DISCUSSION**

In the current study, the effect of lacidipine, TPP, and LTC on experimentally induced OIS in rats was investigated biochemically, and the retinal tissues were examined histopathologically. According to the literature, excessive levels of reactive oxygen species (ROS) produced by mitochondria during the reperfusion process after ischemia may result in oxidative stress. In biological membranes, ROS produced during I/R reacted with unsaturated fats to produce MDA, the end product of LPO. In addition to being a biological marker of tissue damage, MDA was also one of the most important markers of oxidative damage. In this study, it was revealed that there was a significant increase in MDA level in the eye tissue of the I/R group compared to the control group. It was reported in previous studies that ROS...
production in the retinal tissue increased as a result of I/R, inducing LPO and ultimately causing oxidative damage[39]. On the other hand, lacidipine, TPP, and LTC were found to significantly inhibit the increase in MDA levels in the eye tissue induced by I/R. Nevertheless, LTC treatment reduced the LPO reaction intensity to the highest level and brought it closer to the levels observed in the control group. In the literature, there was no information on the possible effects of lacidipine and TPP on MDA levels in the retina following I/R injury. However, it was reported by Khurana et al[7] that lacidipine exerted an antioxidant effect by preventing the increase in LPO in brain tissue. Cinici et al[21] reported that TPP protected the retina of the eye from oxidative damage associated with hyperglycemia by suppressing the production of MDA. On the other hand, it has been reported in the literature that LPO is increased in diabetic cataract lenses and serum MDA levels are increased in patients with diabetic cataract. The potential benefit of lasidipine, TPP and LTC in diabetic cataract is an open field to be studied[22].

As is well known, endogenous antioxidants contributed significantly to the removal of ROS formed during I/R[23]. Therefore, in our study, the level of tGSH, an endogenous antioxidant, was measured. GSH protects the cell against ROS damage[24]. A decrease in GSH levels as a result of I/R was reported to be associated with an increase in LPO in the literature[17]. Wu et al[25] showed that there was a significant decrease in GSH levels in the retinal tissue of rats subjected to I/R, in parallel with the increase in oxidants, compared to the control group. In this study, tGSH levels were significantly decreased in the eye tissue of rats as a result of I/R injury, as has been reported in the literature. LTC, as examined in terms of its ability to prevent oxidative damage, prevented tGSH reduction in eye tissue more significantly than lacidipine or TPP treated alone. In the literature, no information was available regarding the possible effects of lacidipine, TPP, or LTC on tGSH levels in the eye tissue following an I/R injury. There was, however, evidence that lacidipine exerted a protective effect by inhibiting the decrease in GSH in brain tissue[26]. Additionally, literature indicated that TPP protected the retina from oxidative damage associated with hyperglycemia by inhibiting the reduction of tGSH[21].

SOD was another endogenous antioxidant that decreased in the eye tissue of experimental animals as a result of I/R in this study. SOD has been known to reduce superoxide radicals and produce hydrogen peroxide[27]. In contrast, the CAT enzyme prevented the accumulation of hydrogen peroxide[28]. Our biochemical findings revealed that the antioxidant systems in the I/R group were insufficient to neutralize ROS. In the literature, it has been shown that endogenous antioxidant enzymes increase after the increase in ROS in the experimental retinal I/R model[17]. As a result of this study, lacidipine, and TPP significantly inhibited the decrease in SOD and CAT enzyme activity in the eye tissue after I/R, and the activity of these enzymes was the highest in the LTC-applied rats. No other studies examined the effects of lacidipine on SOD and CAT enzymes in eye tissue. However, the role of SOD and CAT enzyme levels in lacidipine’s protective effect was proven[29]. Some studies associated the protective effect of TPP on retinal tissue with SOD and other enzymatic antioxidants[21].

It is known that the increase in calcium ion concentration in retinal I/R pathophysiology is associated with the increase in the production of proinflammatory cytokines[29]. TNF-α was a major inflammatory mediator of neuronal death after ischemic damage to the retina[30]. Studies show that increased proinflammatory cytokines accelerate the inflammatory process

Table 2 Analysis results of histopathological evaluation data obtained from experimental groups

<table>
<thead>
<tr>
<th>Histopathological grading</th>
<th>SG</th>
<th>CCU</th>
<th>LCCU</th>
<th>TCCU</th>
<th>LTCCU</th>
<th>F or H</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal destruction&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>3 (2-3)</td>
<td>1 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1 (1-3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>135.422</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Edema&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>3 (2-3)</td>
<td>1 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1 (0-3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>132.317</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vascular congestion&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>3 (2-3)</td>
<td>0 (0-1)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1 (0-3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>136.271</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Polymorphonuclear cell infiltration&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0&lt;sup&gt;+&lt;/sup&gt;</td>
<td>2 (1-3)</td>
<td>0 (0-1)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1 (0-3)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0 (0-2)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>129.575</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Retina thickness of layers (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner plexiform layer&lt;sup&gt;5&lt;/sup&gt;</td>
<td>109.72±0.79</td>
<td>163.12±1.27</td>
<td>120.88±1.88</td>
<td>136.43±1.54</td>
<td>112.74±0.76</td>
<td>402.349</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inner nuclear layer&lt;sup&gt;5&lt;/sup&gt;</td>
<td>120.48±1.10</td>
<td>157.49±1.22</td>
<td>127.02±1.73</td>
<td>136.82±1.19</td>
<td>121.91±1.70</td>
<td>200.655</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Outer nuclear layer&lt;sup&gt;6&lt;/sup&gt;</td>
<td>163.95 (142.60-186.90)</td>
<td>242.60 (216.40-258.10)</td>
<td>191.65 (180.90-199.40)</td>
<td>217.55 (205.60-241.60)</td>
<td>171.35 (154.20-189.10)</td>
<td>164.078</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total retina&lt;sup&gt;7&lt;/sup&gt;</td>
<td>655.25 (623.50-680.20)</td>
<td>780.95 (731.90-819.00)</td>
<td>712.60 (708.60-722.60)</td>
<td>743.85 (719.50-760.60)</td>
<td>672.80 (663.20-691.00)</td>
<td>167.622</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of ganglion cells in the ganglion cell layer</td>
<td>8 (7-9)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>5 (4-6)</td>
<td>6 (5-7)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>6 (5-6)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>8 (7-9)&lt;sup&gt;9&lt;/sup&gt;</td>
<td>144.985</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>1</sup>P<0.05 vs CCU; <sup>2</sup>P>0.05 vs SG; <sup>3</sup>P>0.05 vs CCU. <sup>4</sup>Kruskal-Wallis test, Dunn’s test, mean±standard error. SG: Sham surgery group; CCU: Right and left common carotid cross-clamping and unclamping; LCCU: Lacidipine+CCU; TCCU: Thiamine pyrophosphate+CCU; LTCCU: Lacidipine+TCCU.
and ultimately negatively affect neuronal survival after retinal I/R[31]. A significant increase was observed in TNF-α, IL-1β, and IL-6 levels in the eye tissues of rats in the I/R-mediated OIS model. These findings were consistent with those obtained in the studies conducted by Wang et al.[32]. LTC prevented the increase of TNF-α, IL-1β, and IL-6 in I/R treated eye tissue more significantly than lacidipine and TPP treated alone. No studies were found in the literature on lacidipine’s effects on proinflammatory cytokine levels in eye tissue. However, Karakus et al.[31] reported that inflammation induced by serum TNF-α, IL-1β, and IL-6 levels were suppressed as a result of lacidipine treatment. It was documented that TPP suppressed the increase of TNF-α, and IL-1β in the optic nerve tissue and protected the optic nerve from cytokine damage[33]. Kundu et al.[34] found that suppression of cytokines such as TNF-α, IL-1β and IL-6 decreased ocular surface inflammation and symptoms in dry eye disease. Oxidative stress has also been held responsible for the pathogenesis of dry eye disease[35]. Lacidipine, TPP and especially LTC have the potential to be beneficial for dry eye disease with their antioxidative and anti-inflammatory activities.

Histopathological findings supported the biochemical findings obtained in this study. Histological analysis revealed that I/R caused serious degeneration of retinal tissue, decrease in retinal tissue thickness, dilation of blood vessels, and enlargement of polymorphonuclear cells, as well as edema and vacuolation. These histopathological findings, such as the thinning of retinal tissue and the decrease in ganglion cells, were supported by histopathological findings in rats with OIS[36,37]. An et al.[38] found a decrease in total retinal thickness, IPL, INL, and OPL. There was even evidence that OIS caused loss of pupillary reflex and death of retinal ganglion cells and photoreceptors[39].

Our histopathological analysis results support the findings of Cinici et al.[31] study showing that TPP reduces oxidative ocular damage.

In conclusion, bilateral common carotid artery ligation caused an increase in oxidant and proinflammatory cytokines and a decrease in antioxidants in the eye tissue of rats. Additionally, OIS-induced histopathological signs were found in the eye retina. Lacidipine, TPP, and LTC significantly prevented the alteration of oxidant, proinflammatory cytokine, and antioxidant parameters in eye tissue with common carotid artery ligation. Additionally, lacidipine, TPP, and LTC significantly reduced OIS symptoms histopathologically. It was LTC>lacidipine>TPP that inhibited the oxidative and histopathological harm associated with carotid artery ligation the best. The results of our experiments indicated that LTC could be more beneficial than lacidipine and TPP alone in treating OIS. In addition to measuring the total levels of oxidants and antioxidants in the eye tissue, a molecular analysis of the histopathology of the eye tissue was recommended.

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