Interleukin-11: A Novel Agent in Retinal Ischemia-Reperfusion Injury

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Abstract

Objective: To investigate the protective effect of recombinant human IL-11 (rhl-11) administered in a systemic manner on retina tissue during ischemia/reperfusion (I/R) injury in a guinea pig model.

Study design/materials and methods: An experimental study in retinal I/R. Placebo, ischemia/sham, ischemia/rhl-11 groups including five animals in each were formed from male albino guinea pigs. Retinal ischemia was induced by cannulating the anterior chambers and lifting the bottle to a height of 205 cm for 90 Min in the sham and ischemia/rhl-11 groups. The ischemia/sham and ischemia/rhl-11 groups received 0.1 cc of a saline solution and 5 μg/kg/day rhIL-11 intraperitoneally one hour before the ischemic insult and during two days of reperfusion, respectively. The guinea pigs were sacrificed for biochemical analysis and the levels of tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and interleukin-6 (IL-6) in retina were analyzed with ELISA. Mann–Whitney-U and Kruskal–Wallis tests were used for statistical analysis.

Results: The mean TNF-α level of the sham group was statistically significantly higher than that of the placebo group (p=0.008). The mean TNF-α levels of the placebo and ischemia/rhl-11 groups were significantly different (p=0.008). The mean retinal TNF-α level of the ischemia/rhl-11 group was statistically significantly lower than that of the sham group (p=0.008). The mean retinal TGF-β levels of the sham group was statistically significantly higher than that of the placebo group (p<0.008). However, the mean retinal TGF-β levels of the placebo and ischemia/rhl-11 groups were not statistically significantly different (p=0.690). The mean retinal TGF-β level of the ischemia/rhl-11 group was statistically significantly lower than that of the sham group (p=0.032). The mean retinal IL-6 level of the sham group was significantly higher than that of the placebo group (p=0.008), while there was no statistically significant difference between the placebo group and ischemia/rhl-11 group for retinal IL-6 levels (p=1.00). The mean retinal IL-6 level of the ischemia/rhl-11 group was significantly lower than that of the sham group (p=0.008).

Conclusion: rhl-11 treatment reduces the levels of TNF-α, IL-6 and TGF-β in the retina of the ischemia/reperfusion-injured guinea pig retinas.

Keywords: Retinal ischemia/reperfusion injury; Recombinant human IL-11; TNF-α; IL-6; TGF-β

Abbreviations: rhIL-11: Recombinant Human Interleukin-11; TNF-α: Tumor Necrosis Factor-α; TGF-β: Transforming Growth Factor-β; IL-6: Interleukin-6

Introduction

The retina is highly sensitive to ischemic injury and reperfusion, both of which inevitably result in cellular damage and death. Reperfusion induces the synthesis of inflammatory mediators, oxygen derived free radicals and lipid mediators, and activation of the bone-marrow born cells [1]. It has been known that the glial cells exposed to ischemia hasten the death of the retinal ganglion cells by TNF-α synthesis [2]. In ischemia-reperfusion damage, the level of IL-6 in the retina has been shown to significantly increase and TGF-β has been shown to be protective [3,4].

Recombinant human IL-11 (rhIL-11) is a mediator that plays a role in hematopoiesis and hemopoiesis and consists of 199 amino acids. The molecular weight of rhIL-11 is 23 kDs. It was first described by Paul et al in 1990 [5]. Recombinant human (rh) IL-11 is produced by recombinant DNA technology in E coli bacteria (Oprelevkin, Neumega, Wyeth, USA). It has been used in the patients with non-myeloid malignancies receiving immnosuppressive chemotherapy over bone marrow and requiring thrombocyte infusion [6].

Moreland et al. have reported that rhIL-11 reduces the production of the proinflammatory cytokines and nitric oxide levels [7]. rhIL-11 shows its protective effect by down regulating the expression of TNF-α and IL-1β, inhibiting the synthesis of nitric oxide, and stimulating cellular proliferation and differentiation [6]. It has also been shown to act via reducing oxidative stress and up-regulating Heat Shock Protein 25 [8]. Another study has shown that it acts by reducing the levels of mRNA of pro inflammatory cytokines such as IFN- γ [9].

The present study aimed to investigate the protective effect of rhIL-11 administered in a systemic manner on retina tissue during ischemia/reperfusion (I/R) injury in a guinea pig model.

Materials and Methods

Experimental protocol

Fifteen male albino guinea pigs with a mean weight of 500 g (range: 470–640g) were used in the study. All the procedures involving animals were conducted in accordance with the guidelines of Association for Research in Vision and Ophthalmology Resolution on the use of Animals in Research. All the animals were housed in individual cages and maintained under standard conditions at the Experimental Research Center of Firat University. The study protocol was approved by the Institutional Animal Care and Use Committee of Firat University.

Groups

Three groups of guinea pigs were established randomly, with five animals in each.

The first group was determined as the placebo group. In this group, no ischemia or reperfusion was induced, and the animals
(n=5) were administered 0.1 cc of a saline solution intraperitoneally as placebo.

The second group was determined as the sham group. In this group, ischemia and reperfusion were induced, and the animals (n=5) were administered a saline solution intraperitoneally as placebo (n=5).

The third group was determined as the ischemia/rhIL-11 group. In this group, ischemia and reperfusion were induced, and the animals (n=5) were administered rhIL-11 were administered intraperitoneally.

**Anesthesia technique**

All the animals were anaesthetized with 50 mg/kg ketamine HCl and 5mg/kg xylazine HCl intramuscularly. Topical anesthetic consisting of 0.005% proparacaine HCl was administered to both eyes of each animal.

**Induction of ischemia and reperfusion**

Both eyes of all the animals were included in study. However, only one eye of the animals in each group was used for biochemical assay.

Pressure-induced retinal ischemia was achieved by cannulating both anterior chambers with a 27-gauge needle connected to a bottle of normal saline and lifting the bottle rapidly to a height of 205 cm in order to raise the intraocular pressure to 150 mm Hg. This lasted for 90 min, and reperfusion was established by lowering the saline bottle to the eye level. Then, the eyes were decannulated. The reperfusion period after ischemia lasted 24hours [10].

In the placebo group, which was not inflicted ischemia and reperfusion, 0.1 cc/day saline solutions was given intraperitoneally for two days. In the sham group, 0.1 cc of a saline solution was given intraperitoneally one hour before the ischemic insult and during two days of reperfusion period. In the ischemia/rhIL-11 group, 5 μg/kg/day Recombinant IL-11 was given intraperitoneally one hour before the ischemic insult and during two days of reperfusion period.

At the end of the second day of the experiment, all the animals were re-anesthetized, and both eyes of all the animals were rapidly enucleated and then, the animals were sacrificed using intracardiac thiopental sodium (50 mg/kg).

**Biochemical assay**

The enucleated eyes were immediately placed on the ice slices. They were then dissected coronally through the pars plana for biochemical assay. After removing the vitreous, the retinal tissue was homogenized at a rate of 16.000 cycles/minute Ultra Turrax T25 Basic homogenizator (IKA Labortechnik, Germany). The supernatants were collected by centrifugation at 3500 rpm for 45 Min, covered in aluminum foil and stored at –80°C until the biochemical assay.

The levels of TNF-α (pg/ml), IL-6 (pg/ml) and TGF-β (pg/ml) in the retina were analyzed with Biosource Rat TNF-α immunoassay kit (Cat. No: KRC3012, USA), Biosource Rat IL-6 ELISA kit (Cat. No: KRC0061, USA), and Biosource Rat TGF-β ELISA kit (Cat. No: KAC1688, USA) in Triturus Grifols (Spain) full-automatic ELISA device.

**Statistical analysis**

Statistical analysis was performed using SPSS version 13.0 (SPSS Inc, Chicago, Illinois, USA). Mann–Whitney–U test was used to evaluate the differences between the groups. Krukskal–Wallis test was used to evaluate the intragroup differences. The data were expressed as mean ± SD. A p value less than 0.05 was considered statistically significant.

**Results**

The mean retinal TNF-α level of the placebo, sham, and ischemia/rhIL-11 groups were 222.80 ± 19.14, 787.80 ± 191.13, 321.60 ± 26.26 pg/ml, respectively. The mean retinal TNF-α level of the sham group was statistically significantly higher than that of the placebo group (p=0.008). The mean retinal TNF-α levels of the placebo and ischemia/rhIL-11 groups were also statistically significantly different (p=0.008). The mean retinal TNF-α level of the ischemia/rhIL-11 group was statistically significantly lower than that of the sham group (p=0.008) (Table 1 and Figure 1).

The mean retinal TGF-β levels of the placebo, sham and ischemia/rhIL-11 groups were 4640.0 ± 385.12, 7048.0 ± 1201.46, 4944.0 ± 784.14 pg/ml, respectively. The mean retinal TGF-β level of the sham group was significantly higher than that of the placebo group (p=0.008) (Table 1 and Figure 1).

**Table 1: The levels of retinal TNF-α, TGF-β and IL-6 in the study groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (pg/ml) Mean ± SD (Range)</th>
<th>TGF-β (pg/ml) Mean ± SD (Range)</th>
<th>IL-6 (pg/ml) Mean ± SD (Range)</th>
</tr>
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<tbody>
<tr>
<td>Placebo</td>
<td>222.80 ± 19.14 (194-247)</td>
<td>4640.0 ± 385.12 (4640-5600)</td>
<td>37.90 ± 7.62 (29.6-48.4)</td>
</tr>
<tr>
<td>Sham</td>
<td>787.80 ± 191.13 (467-980)</td>
<td>7048.0 ± 1201.46 (5600-8960)</td>
<td>148.70 ± 38.99 (86.9-194)</td>
</tr>
<tr>
<td>Ischemia/rhIL-11</td>
<td>321.60 ± 26.26 (289-361)</td>
<td>4944.0 ± 784.14 (4160-6240)</td>
<td>43.46 ± 19.78*** (27.1-69.4)</td>
</tr>
</tbody>
</table>

Notes:

* p=0.008 for the sham group vs the placebo group; ** p=0.008 for the ischemia/rhIL-11 group vs the sham group (p=0.008); *** p=0.008 for the sham group vs the placebo group; **** p=0.008 for the ischemia/rhIL-11 group vs the sham group.

**Figure 1:** The comparison of the retinal TNF-α levels among the study groups* p<0.05, statistically significant difference.
(p=0.008). However, there was no statistically significant difference between the placebo group and ischemia/rHIL-11 group for retinal TGF-β level (p=0.690). The mean retinal TGF-β level of the ischemia/rHIL-11 group was statistically significantly lower than that of the sham group (p=0.032) (Table 1 and Figure 2).

The mean retinal IL-6 levels of the placebo, sham, and ischemia/rHIL-11 groups were 37.90 ± 7.62, 148.70 ± 38.99, 43.46 ± 19.78 pg/ml, respectively. The mean retinal IL-6 level of the sham group was significantly higher than that of the placebo group (p=0.008), while the mean retinal IL-6 levels of the placebo and ischemia/rHIL-11 groups were not statistically significantly different (p=1.00). The mean retinal IL-6 level of the ischemia/rHIL-11 group was statistically significantly lower than that of the sham group (p=0.008) (Table 1 and Figure 3).

Discussion

Retinal ischemia is one of the most common causes of loss of vision and blindness. Ischemia plays a crucial role in vaso-occlusive and vaso-proliferative diseases of the retina. Diabetes mellitus, retinal artery occlusion, retinal vein occlusion, retinopathy of prematurity, sickle cell anemia, and ocular inflammatory disorders commonly cause the ischemic injury in the retina [11].

Numerous studies have demonstrated that free radicals play a role in retinal ischemic injury [1,2,12]. They cause tissue injury through lipid peroxidation in the cell membranes, alteration in the structure of the proteins, carbohydrates, nucleic acids, and DNA, disturbance of calcium balance and stimulation of the release of excitatory amino acids like aspartate and glutamate [12,13]. Yoneda et al. have determined that IL-1β plays a role in ischemia-reperfusion injury in a rat model and that receptor antagonists of IL-1β prevent ischemic injury [14]. Additionally, the levels of IL-1β, TNF-α, IFN-γ, TGF-β and IL-6 have been reported to increase in IR injury and that IL-1β mediates the degeneration in the inner retinal layers [15].

TNF-α is a messenger inducing the apoptosis in retinal ganglion cells. Although TNF-α at picogram levels is considered non-cytotoxic, it induces cell death by ceasing the survival signals during neurodegeneration. Additionally, it has been known that TNF-α is also responsible for the glial changes and axonal degeneration in the optic nerve, which is observed in patients with AIDS [16]. TNF-α increases the production of the nitric oxide via induction of nitric oxide synthetase in astrocytes. Nitric oxide causes lipid peroxidation in the cell membrane by interacting with O₂ radical and peroxynitrite in IRI [17]. Fontaine et al. have found that the activation of TNF-α receptor 1 promotes the cell death and that the activation of TNF-α receptor 2 has neuro protective effects when mouse retina is exposed to IRI [18]. In our study, after IRI of the retina, it was found that the levels of TNF-α in the sham group were significantly higher than those of the placebo group (p=0.008). TNF-α is known to be a potent inflammatory cytokine following ischemic retinal injury. It is released by reactive macrophages, astrocytes, microglia and retinal glial cells during the inflammatory process. Following the injury in IR, the release of TNF-α stimulates nitric oxide and excitotoxins and consequently plays a role in ischemic injury [2,19]. TNF-α increases the production of nitric oxide by inducing nitric oxide synthetase. Nitric oxide interacts with superoxide and peroxy nitrite radicals, and thus disrupts the structure of the cell membrane by lipid peroxidation, thereby causing ischemic injury [20].

In another study evaluating the astrocyte cell culture in human lamina cribrosa, Yu et al were the first to find that the levels of αB-crystalline, TGF-β1 and TGF-β2 were elevated in the ischemia-reperfusion process. Additionally, they demonstrated that the usage of TGF-β neutralizing antibodies down regulates the levels of αB-crystalline and protects the cells from IRI [21]. Welge-Lussen et al. demonstrated that the levels of αB-crystalline were increased via the stimulation by TGF-β in the cells of trabecular meshwork and ciliary muscle. Thus, it has been considered that TGF-β1 and TGF-β2 are potent inducers of αB-crystalline, a heat shock protein, all of which may be responsible for IRI [22]. In our study, TGF-β levels of the sham group were significantly higher than those of the placebo group. In a recent study, it has been demonstrated that the levels of αB-crystalline were increased following the period of cerebral ischemia-reperfusion [21]. In another study, an increase in the synthesis of mRNA and protein of αB-crystalline has been observed in the astrocytes of the optic nerve head during reperfusion process. This molecule is a heat shock protein that generates after ischemia [22]. In the glial cells and neurons in the central nerve system, HSP70 and HSP27 molecules provide the expression of mRNA and protein and consequently, the expression of TGF-β1 during the period of ischemia and reperfusion. TGF-β contributes to IRI process by increasing the production of reactive oxygen radicals. Additionally, it was determined that other factors mediating IR also increased the intensity of ischemic injury in the optic nerve head by increasing the expression of TGF-β [21]. Decreasing in the TGF-β levels by rhIL-11 might reduce the levels of
αB-crystalline and other heat shock proteins and might protect the cells from IRI.

It has been shown that the protein and mRNA of Endogenic IL-6 increase following IR. These data support that increase in the endogenic IL-6 is a defense mechanism of inner retinal layers including retinal ganglion cells after IRI. In addition, microglial-phagositic cells play an important role in this mechanism [3].

Fischer et al. have demonstrated that IL-6 might augment the ischemic injury [23]. In our study, it was determined that the mean IL-6 level of the sham group was significantly higher than that of the placebo group (0.008). Sappington et al. showed that the expression of Bax and Bcl-2 proteins inducing apoptosis increased in the retinal ganglion cells during ischemic injury. The increase in the Bax and Bcl-2 proteins play a role in injury to retinal ganglion cells that is induced by high-pressure [24]. Previous studies have shown that nitric oxide and TNF-α released by glial cells accelerate the death of the retinal ganglion cells [25]. IL-6 has been shown to affect the lifetime of the ganglion cells by regulating the Bax and Bcl-2 gene expressions [26].

An earlier study evaluating rhIL-11 demonstrated that the levels of TNF-α, IL-6 and IL-1β increased following intestinal ischemia and that the levels of these cytokines were reduced by rhIL-11. A possible mechanism of this condition has been suggested as increased MHC Class I and II antigen presenting cells in intestinal diseases, and it has also been suggested that these cells may be inhibited by IL-11. Another possible mechanism is the modulation of IFN-γ gene expression by NFκB, thereby suppressing the levels of the inflammatory cytokines such as TNF-α, IL-1β, IL-6 [27]. In another study, it was reported that the levels of TGF-β reduced and the levels of IFN-γ decreased via the increase in the levels of IL-11. Similarly, it was emphasized that IL-11 decreases the levels of GM-CSF [28].

In our study, while the levels of TNF-α of the ischemia/rhIL-11 group were significantly higher those of the placebo group, no statistically significant difference was determined between the TGF-β and IL-6 levels. The levels of TNF-α, TGF-β and IL-6 of the ischemia/rhIL-11 group were significantly lower than those of the sham group. RhlIL-11 also inhibits the release of IFN-γ and IL-2 by activate T cells [29]. In an earlier study, it was demonstrated that IL-11 suppressed inflammation and reduced the levels of IFN-γ, TNF-α, IL-1β, IL-6, which are pro inflammatory the cytokines, and inducible nitric oxide synthetase in the colons of the rats with chronic colitis [29].

In conclusion, although the small sample size is a limitation in our study, we believe that IL-11, an anti-inflammatory cytokine, has an important role in IRI and that rh-IL is an agent that can be used in the treatment of ischemic retinal diseases because this drug reduces the levels of TNF-α, TGF-β and IL-6 significantly in ischemic retinal injury. However, further clinical and experimental studies are need for the use of rh-IL11 in clinical practice in patients with retinal disease.

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