Can Endoplasmic Reticulum Stress Inhibition Reduce Inflammation Due to Alkaline Corneal Burns? -Preliminary Report-

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ABSTRACT

Purpose: The role of endoplasmic reticulum stress has not yet been investigated in corneal inflammation. In this preliminary study, we aimed to find out whether endoplasmic reticulum stress inhibition has an effect on inflammation and neovascularization after alkaline burn and to find the effective dose.

Materials and Methods: Salubrinal was used as an endoplasmic reticulum stress inhibitor. The study was carried out on Wistar rats of 250-300 mg divided into four groups, with 1 animal in each group. After the corneal neovascularization (CNV) model was created in the right eyes, subconjunctival salubrinal was administered at doses of 0.1; 0.25; 0.5 and 1 mg/kg respectively. Subconjunctival Salubrinal was applied to the left eyes without creating a CNV model. Histopathological results were evaluated in the corneal tissues taken from animals that were sacrificed on the 14th day.

Results: As a result of histopathological evaluation performed in 4 groups, inflammation and vascularization were observed in the groups given 0.1 mg / kg, 0.25 mg / kg and 0.5 mg / kg salubrinal, respectively. In the group given 1 mg / kg salurinal, it was observed that inflammation was less than the other groups and there was no vascular structures. In any group, salubrinal did not show toxic effects on intact corneal tissue histologically.

Discussion: Inhibition of endoplasmic reticulum stress, which is a pathway of inflammation, can be used in the treatment of inflammation and corneal neovascularization caused by alkaline burns. More comprehensive and comparative studies should be conducted on this subject.

Keywords: Endoplasmic Reticulum Stress, Salubrinal, Neovascularisation, Alkaline corneal burn, Corneal Inflammation.

INTRODUCTION

The endoplasmic reticulum (ER) is recognized as the organelle responsible for protein synthesis and folding in basically all eukaryotic cells. On the other hand, ER stress is the formation of unfolded or misfolded proteins and their accumulation in the ER lumen as a result of physiological or pathological stimuli that cause an imbalance between the protein folding load and the capacity of the ER. As a result, this accumulation activates the unfolded protein response (UPR) to provide homeostasis.[1] Previous studies in the literature, show that there is an extensive correlation between the inflammatory response and the ER stress response.[2,3] UPR and inflammation are connected with a variety of mechanisms, such as the production of acute phase reactants and reactive oxygen species (ROS), activation of the transcription factor nuclear factor-κB (NF-κB). The role of ER stress has not yet been investigated in corneal inflammation, although it has been previously studied in a retinal cell injury model.[4]

Salubrinal is an endoplasmic reticulum stress inhibitor described by Boyce et al. that has been shown to inhibit alpha dephosphorylation of the eukaryotic translation initiation factor (EIF2α).[5] Phosphorylation of EIF2α exerts a cytoprotective effect by reducing protein synthesis during ER stress.[6] In this preliminary study, we aimed to find out whether endoplasmic reticulum stress inhibition has an effect on inflammation and neovascularization after alkaline burn and to find the effective dose of *salubrinal*.

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MATERIALS AND METHODS:

The study was conducted with the permission granted by the Suleyman Demirel University Ethics Committee to conduct research on animal subjects (28.03.2019-05/03). All experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health. The study was planned as a preliminary study to investigate the probable effect and to find the effective dose. The study was carried out on Wistar rats of 250-300 mg divided into four groups, with one animal in each group. Rats were placed in an all-day controlled room for temperature (21–22 °C), humidity (60±5%) and 12:12 h light/dark cycle. All animals were fed with standard commercial chow diet.

After intraperitoneal ketamine (90 mg/kg) (Putney Inc, Portland, ME), and xylazine (20 mg/kg) (Vedco Inc, St Joseph,MO) anesthesia, both eyes of the animals in the group were examined and a chemical burn of approximately 2 mm in width was created by cautery for 4 seconds with a rod coated with 75% silver nitrate and 25% potassium nitrate in the corneal center of the right eyes.[7] *Salubrinal* was used as an endoplasmic reticulum stress inhibitor. Immediately after the corneal neovascularization (CNV) model was created in the right eye, subconjunctival salubrinal was administered at doses of 0.1, 0.25, 0.5 and 1 mg/kg, respectively. *Salubrinal* was not administered systemically. The dose selection for local use was planned as four different doses, each one half as much as the previous one, with *salubrinal* systemic dose of 1 mg / kg. Subconjunctival *salubrinal* was applied to the left eyes without creating a CNV model. Topical tobramycin was administered qid till sacrification. Histopathological results were evaluated in corneal tissues taken from animals sacrificed on the 14th day.

RESULTS

At the end of the study, as a result of the histopathological evaluation performed in 4 groups, inflammation and vascularization were observed, although decreased in the groups given 0.1 mg / kg 0.25 mg / kg and 0.5 mg / kg *salubrinal*, respectively. (Figure 1A-1C) In the group given 1 mg / kg salubrinal it was observed that inflammation was less than the other groups and there were no vascular

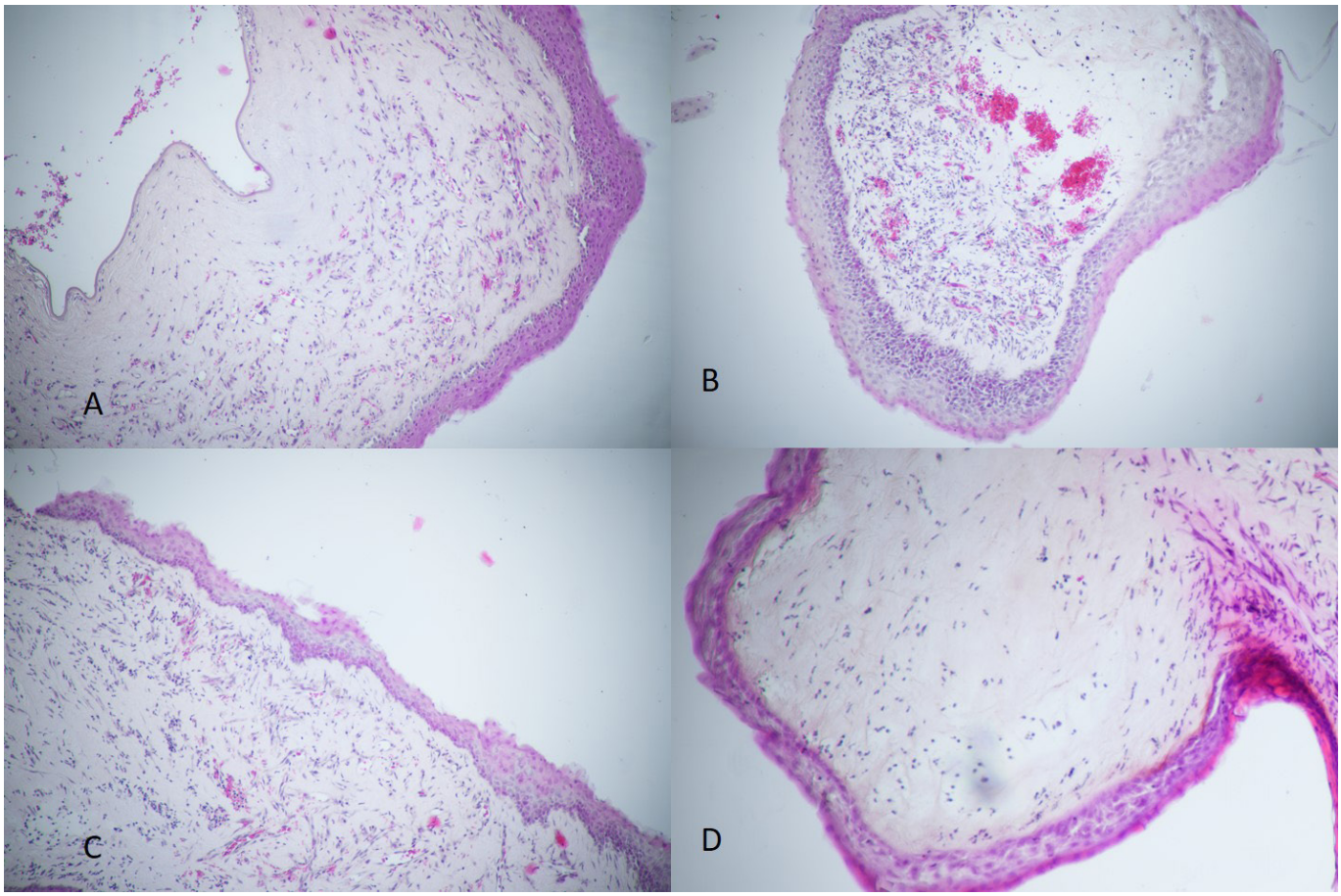

Figure 1: A-C: *Histopathological evaluation of the groups 0.1 mg / kg, 0.25 mg / kg and 0.5 mg / kg salubrinal, respectively. Inflammation and vascularization were observed.* ***D:*** *It was observed that in the group given 1 mg / kg salubrinal, inflammation was much less than the other groups and there were no vascular structures.* (H-E, Scale bar 50 µm, 40x)

structures. (Figure 1D) In any group, *salubrinal* did not show toxic effects on intact corneal tissue histologically. (Figure 2)

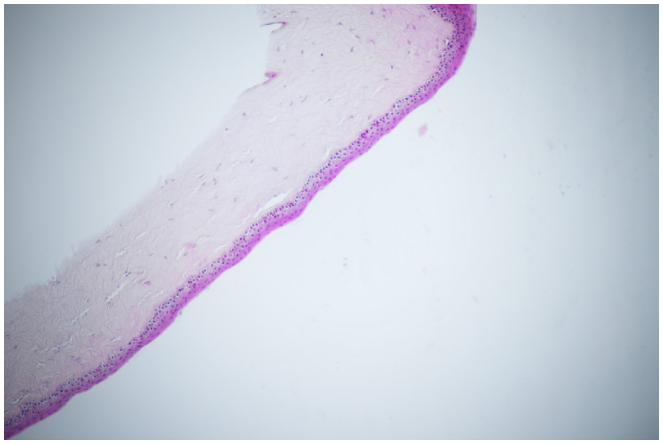

Figure 2: *Salubrinal did not show toxic effects on intact corneal tissue histologically.* (H-E, Scale bar 50 µm, 40x)

DISCUSSION

In this study, we first aimed to investigate whether salubrinal, an endoplasmic reticulum stress inhibitor, has an effect on neovascularization after corneal alkali burn. For this purpose, we tried to find the lowest subconjunctival dose that could have an effect, but did not show toxic effects, by choosing three different doses together with the systemically applied dose of 1 mg / kg in rats with an alkaline burn model.[8] We attained the best result with a dose of 1 mg / kg and we found that it had no toxic effect in topical application.

The ER stress due to inflammatory factors such as hypoxia or viral infections harms the cellular activities by diminishing the protein folding function.[9] In this process; phosphorylation of the alpha subunit of eukaryotic initiation factor 2a (eIF2a) activates to induce cell apoptotic cell death. Consequently, modulation of a phosphorylated eIF2a level potentially alters the fate of damaged tissues.[10] *Salubrinal* has been identified as a compound capable of increasing eIF2a phosphorylation, inhibiting ER stress and protecting cells from ER stress-induced apoptosis.[5]

Inflammation is the basic mechanism of CNV caused by alkaline corneal burns.[11] Angiogenic growth factors (VEGF family) are released as an end product by the activation of inflammatory cells such as macrophages and neutrophils. The main reason for this triggering initiated by inflammatory cells during corneal injury is to support limbal vascular endothelial cell proliferation and migration.[12] Interleukin-1 (IL-1) released mainly from damaged corneal epithelium; has been shown to stimulate inflammatory angiogenesis and direct proliferation and migration of endothelial cells, thereby being a pro-inflammatory cytokine that supports CNV. In addition, IL-8, IL-17A and matrix metalloproteinases have an important role in corneal angiogenesis.[13]

The role of ER-Stress pathway were studied on retinal and trabecular meshwork cells before.[4,14-16] Li et al demonstrated reactive oxygene radical overproduction and ER stress activation with all trans retinal induced damage model in retina pigment epithelium cells. Induced damage resulted in cellular mitochondrial dysfunction and apoptosis of retinal pigment epithelial cells, which could be blocked by N acetyl cysteine, a potent antioxidant.[14] Similarly, Wang et al. suggested that there could be a novel stem cell therapy model that would regulate the PERK signaling pathway to preserve or regenerate trabecular meshwork cells.[15] Woodward et al. revealed the role of ER stress in the pathophysiologic pathway of the autoimmune ocular surface disease.[17] Another study revealed that salubrinal protects endoplasmic reticulum stress-associated apoptosis in human lens epithelial cells.[18] However, the role of endoplasmic reticulum stress in the inflammatory pathway after corneal injury has not been studied yet.

CONCLUSION

Inhibition of endoplasmic reticulum stress, which is a pathway of inflammation, can be used in the treatment of inflammation and corneal neovascularization caused by alkaline burns. This study is a preliminary study showing that salubrinal can be effective at a dose of 1 mg / kg in corneal inflammation. The results of larger animal studies comparing salubrinal with an anti VEGF will be more enlightening.

Conflict of interest: The authors declare that they have no competing interest.

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