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Curative effect and possible mechanism of taurine on early corneal alkali burns

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To the Editor: Corneal alkali burns (CABs) have been a difficult problem in clinical treatment for a long time. In severe cases, blindness occurs; hence, research on the repair mechanism underlying corneal injury is particularly important to identify potential therapeutic targets. Alkali-induced corneal injuries often trigger aggressive aseptic inflammatory responses, which are key to autoimmune responses.[1] However, Nod-like receptor protein 3 (NLRP3) inflammasomes play an important role in aseptic inflammation.[2] Under stimuli, such as infection or stress, the activated NLRP3 inflammasome not only processes pro-caspase-1 into mature caspase-1 but also further promotes the maturation and release of interleukin (IL)-1β and IL-18.[3] Taurine (Tau) is widely distributed in ocular structures and is an immune regulator. Tau can maintain the stability of the corneal epithelium and improve the survival rate of corneal epithelial cells through antioxidant effects.[4] The purpose of this study was to investigate the effect of Tau eye drops on CAB and elucidate the mechanism underlying corneal inflammatory responses.

We established a C57BL/6 mouse model of CABs (1N NaOH soaked filter paper, 10 s). This experiment was approved by the Ethics Committee of Animal Experiments of Xiangyang No.1 People's Hospital, Hubei University of Medicine (No: 2020DW003). The mice ($n = 35$) were randomly divided into three groups: normal (healthy cornea, $n = 5$), PBS (PBS + CAB, $n = 5$/time point), and Tau (Tau + CAB, $n = 5$/time point). PBS and Tau (concentration = 5%; Wujing Pharmaceutical Co., Ltd, Wuhan, China) were all topically administered (eye drops). The corneas in each group of mice were photographed on day 5 post-burn injury [Figure 1A]. Corneal opacity scores (0, normal; 1, mild hazy, visible iris; 2, moderate hazy, iris still detectable; 3, severe hazy, iris is difficult to detect; and 4, completely obscure, iris is not visible) were higher in the PBS group than in the Tau group at 1 to 5 days after burns ($P < 0.05$) [Figure 1B]. Healthy corneal tissue is intact and of normal thickness. In hematoxylin-eosin staining [Figure 1C], the PBS group showed complete corneal epithelium defects at 2 days and gradual growth at 5 days, but the structure was still disorganized and sparse. The corneal epithelium of the Tau group was partially damaged and disorganized at 2 days and thinned but relatively orderly at 5 days. The optical density of inflammatory factors (NLRP3, IL-1β, and IL-18) in the PBS group was enhanced compared with that in the Tau group at 5 days post-burn injury as determined by immunohistochemistry (IHC). The signals were localized predominantly in the corneal epithelium and slightly in the corneal stroma [Figure 1D] ($P < 0.05$).

At 2 days post-burn injury, the corneal structures of the normal group were intact, but the fluorescence intensity was the weakest, as determined by immunofluorescence staining (IF), and the fluorescence intensity of factors in the corneal tissue of the PBS group was the strongest [Figure 1E]. In the PBS group, the fluorescence intensity of NLRP3 to normal was 12.20 ± 0.90 ($P = 0.0006$); the intensity of IL-18 to normal was 11.39 ± 0.77 ($P = 0.0004$); and the intensity of IL-1β to normal was 10.13 ± 0.67 ($P = 0.0003$) at 2 days after CAB. In the Tau group, the relative fluorescence intensity of NLRP3, IL-1β, and IL-18 was significantly lower than that of the PBS group (NLRP3: 6.33 ± 0.44, $P = 0.0017$; IL-18: 8.64 ± 0.66, $P = 0.0012$ and IL-1β: 8.00 ± 1.04, $P = 0.0037$) at 2 days after CAB but was slightly higher than that of the normal group ($P < 0.05$).

We designed primers for real-time quantitative polymerase chain reaction (RT-qPCR) experiments [Table 1]. The messenger RNA (mRNA) expressions of NLRP3, IL-1β, and IL-18 were normalized to GAPDH. The relative mRNA expression levels of NLRP3 compared with normal were 4.81 ± 0.18 ($P = 0.0003$) and 2.45 ± 0.25 ($P = 0.0004$) in the PBS group at 2 and 5 days, respectively. The relative mRNA expression levels of IL-1β compared with normal were 3.62 ± 0.09 (P = 0.0003) and 1.67 ± 0.22 ($P = 0.0028$) in the PBS group at 2 and

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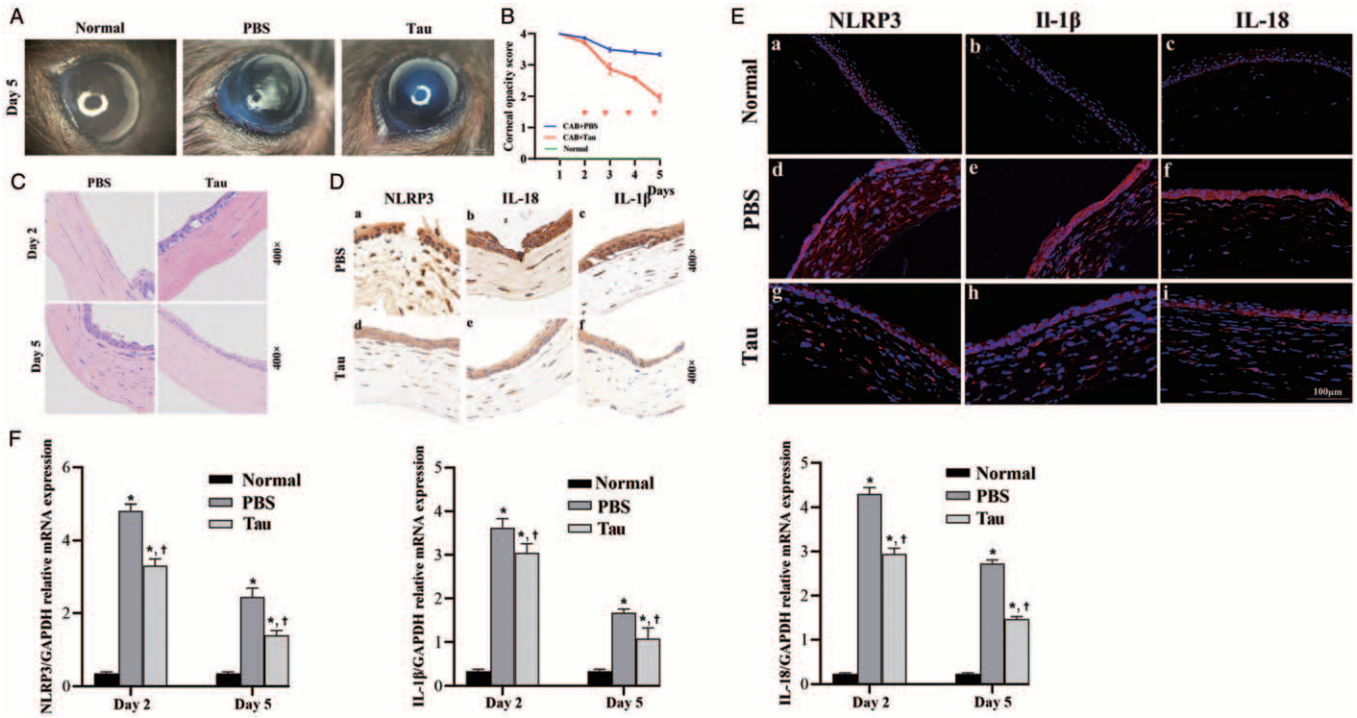

Figure 1: Tau alleviates corneal Injury In CABs by Inhibiting the NLRP3 signaling pathway. (A) Murine corneas In each group at 5 days after modeling (scale: 1000 μm). (B) The corneal opacity scores of each group from day 1 to day 5 after modeling $^{*}P < 0.05$ compared with PBS. (C) Hematoxylin-eosin staining of the PBS group and Tau group at 2 and 5 days after modeling, respectively (scale: 400 ×). (D) The optical density of NLRP3, IL-1β, and IL-18 in the PBS group and Tau group at 5 days post-burn injury via immunohistochemistry (scale: 400 ×). (E) The fluorescence intensity of NLRP3, IL-1β, and IL-18 in each group at 2 days after modeling (scale: 100 μm). (F) The relative mRNA expression levels of NLRP3, IL-1β, and IL-18 in the corneas of each group at 2 and 5 days after modeling $^{*}P < 0.01$ compared with normal; $^{\dagger}P < 0.05$ compared with PBS. CABs: Corneal alkali burns; IL: Interleukin; NLRP3: Nod-like receptor protein 3; Tau: Taurine.

Table 1: Primers used for real-time RT-PCR.

Species-gene-forward/reverse	Sequence of primers (5′-3′)
Mus-Nlrp3-F	CAAGGCTGCTATCTGGAGGAA
Mus-Nlrp3-R	TGCAACGGACACTCGTCATC
Mus-IL-1β-F	GCAGTGGTTCGAGGCCTAAT
Mus-IL-1β-R	GCTGCTTCAGACACTTGCAC
Mus-IL18-F	ACTTTGGCCGACTTCACTGT
Mus-IL18-R	ACAGGCGAGGTCATCACAAG
Mus-GAPDH-F	AACTTTGGCATTGTGGAAGG
Mus-GAPDH-R	ACACATTGGGGGTAGGAACA

RT-PCR: Reverse transcription polymerase chain reaction.

5 days, respectively. The relative mRNA expression levels of IL-18 compared with normal were 4.30 ± 0.15 ($P = 0.0003$) and 2.73 ± 0.29 ($P = 0.0009$) in the PBS group at 2 and 5 days, respectively. The mRNA expression of NLRP3, IL-1 β, and IL-18 in the Tau group was downregulated by RT-qPCR analysis. The relative mRNA expression level of NLRP3 was 3.31 ± 0.18 ($P_{\text{NLRP3 } vs. \text{ normal}} = 0.0007$; $P_{\text{NLRP3 } vs. \text{ PBS}} = 0.0024$), IL-1β was 3.04 ± 0.22 ($P_{\text{IL-1β } vs. \text{ normal}} = 0.0005$; $P_{\text{IL-1β } vs. \text{ PBS}} = 0.0241$), and IL-18 was 3.00 ± 0.13 ($P_{\text{IL-18 } vs. \text{ normal}} = 0.0003$; $P_{\text{IL-18 } vs. \text{ PBS}} = 0.0032$) at 2 days post-burn injury in the Tau group. The relative level of NLRP3 was 1.39 ± 0.13 ($P_{\text{NLRP3 } vs. \text{ normal}} = 0.0027$; $P_{\text{NLRP3 } vs. \text{ PBS}} = 0.0029$), IL-1β was 1.08 ± 0.25 ($P_{\text{IL-1β } vs. \text{ normal}} = 0.0038$; $P_{\text{IL-1β } vs \text{ PBS}} = 0.0052$), and IL-18 was 1.47 ± 0.06 ($P_{\text{IL-18 } vs. \text{ normal}} = 0.0016$; $P_{\text{IL-18 } vs. \text{ PBS}} = 0.0014$) at 5 days post-burn injury in the Tau group. The RT-qPCR results showed the same trends as the IHC and IF findings. The factor expression trends at each time point in each group were consistent ($P < 0.05$); however, the overall expression of factors in each group at 5 days post-burn injury was weaker than that at 2 days [Figure 1F].

In this study, we demonstrated that NLRP3, IL-1β, and IL-18 are involved in the immune response to corneal injury after alkali burns. However, Tau can reduce the damage to corneal tissue by inhibiting the aseptic inflammatory response. CAB is a non-infectious corneal injury, and the body's autoimmune response plays an important role. Early treatment is critical for CABs. In previous alkali burn models, the expression intensity of the factors was correlated with the time of assessment, animal strain, number of samples, and burn duration. In this study, the expression of NLRP3, IL-1β, and IL-18 was detected at 2 and 5 days post-burn injury. We found that the expression of various factors in corneal tissue was upregulated after burn injury and that the expression of the target factors was stronger at 2 days than at 5 days post-burn injury, possibly because of the expression of the NLRP3 inflammasome was influenced by time. This finding is consistent with previous experimental results.[2]

Tau is an essential substance in the human body and is also involved in such processes as antioxidative stress, anti-inflammation, osmotic protection, and regeneration.[4] In the field of ophthalmology, Tau is one of the most

abundant amino acids in the cornea, retina, and lens.[5] The effects of Tau on the retina have been widely studied, but there have been fewer studies on the cornea, and the expression and activity of Tau transporters in human corneal epithelial cells. Tau can improve the survival rate of corneal epithelial cells by maintaining membrane stability and antioxidation. In an infectious model of the porcine cornea,[6] N-chlorotaurine protects the cornea by inactivating *Acanthamoeba* and *Candida albicans* in corneal tissue. In this study, post-burn corneal epithelium injury was repaired to a certain extent by topical treatment with Tau.

To conclude, we showed that Tau can protect the corneal epithelium by reversing the expression of NLRP3, IL-1β, and IL-18. Tau restrained the expression of NLRP3 and then decreased the secretion of IL-18 and IL-1β, which provided novel data on the mechanism and treatment of alkali burn of the cornea. From here, it is necessary to explore further the optimal concentration and treatment duration of Tau and the deeper mechanism of action in corneal diseases.

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Conflicts of interest

None.

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