Original Article

Effect of NF-κB Inhibitor PDTC on a Herpetic Stromal Keratitis Mouse Model

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Abstract

Purpose: To investigate the effect of NF-κB inhibitor PDTC on inflammation response in mice with herpetic stromal keratitis (HSK).

Methods: A total of 120 female BALB/c mice, aged 4-6 weeks, were treated by scuffing the epithelium of the right cornea with the tip of a 30G syringe needle, and approximately 1×10^6 PFU of HSV-1virus was seeded onto the corneal surface. The eye was then exposed to 170 mJ/cm^2 of UV light 7 weeks later, in order to induce the relapse of HSK. PDTC eye drops were adjusted to various concentrations (0.1, 1.0, and 10 mg/ml) with PBS; PBS only was used on control animals. All animals received PDTC or PBS eye drops 6 times/day, 1 drop/time, for 7 consecutive days. Mice were sacrificed at 8h, and 1, 3, and 7d after administration. Corneal tissues were prepared for the detection of IL-1β, IL-4, IL-6, and IL-12 by ELISA and histological study.

Results: Neutrophilic leukocytes and lymphocytes were found in the corneas of HSK mice. ELISA results showed fluctuating expressions of IL-1β, IL-4, IL-6, and IL-12. The IL-1β and IL-4 expressions were significantly lower in the high dose group than in the control group ($P<0.05$), while IL-6 and IL-12 expressions were significantly higher in the high dose group than in the control group ($P<0.05$).

Conclusion: A high concentration of PDTC can suppress the expression of certain inflammatory factors in HSK mice while improving the expression of others. (***Eye Science 2012*; *27*: *188-192***)

Keywords: herpetic stromal keratitis; inflammatory factor; mice

Herpetic stromal keratitis (HSK) is a common infectious corneal disease induced by HSV-1, which leads to corneal scarring, thinning, and vascularization, and can even cause corneal perforation[1]. HSK is highly resistant to treatment with antiviral agents. In addition, the serious adverse events of hormone therapy restrict the long-term usage of antiviral drugs in HSK. The availability of donor corneas for keratoplasty is also limited. Consequently, HSK is characterized by refractory relapse, frequent metastasis, and poor prognosis, which severely affects the quality of life for HSK patients. Therefore, the underlying mechanism of HSK should be explored in order to seek effective drugs and novel targets for HSK therapy. The aim of this study was to investigate the role of PDTC(Ammonium pyrrolidinedithiocarbamate), an inhibitor of NF-κB, in suppression of the inflammation associated with HSK.

Materials and methods

HSV-1 was purchased from ATCC, stored at -80℃. HSV-1 was seeded on ATCC1544 cell lines. HSV-1 was inoculated into Vero cell lines and viral solution was collected 48 h later, following three cycles of freezing and thawing. The supernatant was obtained after centrifugation, diluted at 1:3 ratio to PBS, and re-inoculated into vero cells. After two cell passages and three cycles of freezing and thawing, the supernatant was collected after centrifugation. The viral titer was determined and virus was separately packed for subsequent inoculation.

Establishment of HSK animal models

According to Stuart's method[1], a total of 120 female BALB/c mice (aged 4 to 6 weeks) were used in this study. For virus inoculation, the mice were anesthetized using 0.004 ml/g of 10% chloral hydrate, a decussation-shaped incision was made on the surface of the right eye cornea using a sterile

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30G syringe needle under a microscope. A 5 μl volume of DMEM containing 1×10^6 PFU HSV-1 was administered to the corneal surface, the eyes were kept open for 10 s, and then the eyelids were gently rubbed for 30 s. During the latent infection stage, the CPE assay was used to evaluate the viral infection. After 7 weeks after inoculation, smooth and transparent corneas were exposed to UV-b radiation (170 mJ/cm^2 energy at 302 nm wavelength) to induce the relapse of HSK. The corneal changes and vascularization of the mice with HSK were observed and evaluated.

Drug administration

The HSK mice were randomly divided into 4 groups (low, medium, and high dose groups, and PBS control)and received administration of 0.1, 1.0, and 10 mg/ml PDTC and PBS, respectively. The eyedrops were administered in the right eyes after UV-b radiation treatment, 6 times/d, 1 drop/time, for consecutive 7 days.

Corneal pathological injuries

The mice were sacrificed after anesthesia. The eyeballs were removed, stored in cooling PBS, and the corneal tissues were excised. Half of the corneal tissues were prepared for H.E staining. The number of neutrophilic leukocytes and lymphocytes was counted in three randomly chosen visual fields.

ELISA detection of cytokine levels

The other half of the mouse corneas were kept in 600 μl of serum-free RPMI1640, stored at −80℃ until use. The samples were thawed and homogenized, disrupted by ultrasonication for 30 s, and centrifuged at 1500 r/min for 10 min. The supernatants were subjected to ELISA for the detection of IL-1β, IL-4, IL-6, and IL-12 levels at 8h, and 1, 3, and 7 d.

Statistical analysis

The data from ELISA were analyzed using SPSS 13.0 for statistical analysis. The levels of inflammatory factors among 4 groups were compared using an independent *t*-test $P<0.05$ was considered statistically significant.

Results

Clinical symptoms

Two days after HSV-1 infection, mice showed typical epithelial lesions and corneal epithelitis for 5−7 days. As the epithelial lesions tended to cure, the corneal stromal lesions worsened, showing inflammatory infiltration, edema, corneal neovascularization in the stroma, and even keratohelcosis.

Infiltration of inflammatory cells

H.E. staining revealed that the number of neutrophilic leukocyte peaked at 8 h after the presence of corneal injuries and decreased subsequently. However, the level of lymphocytes was low but consistent (Table 1).

Detection of cytokines

ELISA revealed a peak of IL-1β at 3 h after PDTC treatment in the control, low dose and medium dose groups. The expression level of IL-1β was lower in the medium dose group than in the control group at 8 h, while higher in the low and medium dose

Table 1 The infiltration of inflammatory cells in mice after HSV-1 inoculation

	PBS control		Low dose group		Medium dose group		High dose group	
	N	L	N	L	N	L	N	L
8 h	7.65±16.79	0.76±0.83	1.00±1.00	0.33±0.58	5.80±7.73	0.00±0.00	0.00±0.00	0.44±0.73
1 d	0.07±0.27	0.14±0.53	0.67±0.52	1.00±0.63	7.80±21.56	0.70±1.34	0.00±0.00	0.00±0.00
3 d	2.55±6.56	0.91±1.04	0.00±0.00	0.33±0.52	20.00±29.99	3.63±4.66	8.75±20.04	1.25±1.66
7 d	3.74±7.36	0.68±1.29	0.50±0.93	0.67±1.32	7.33±7.89	2.50±2.59	3.36±6.87	0.64±1.03

Table 2 Changes of IL−1β after drug administration

IL-1β	PBS control	Low dose group	Medium dose group	High dose group
8 h	490.24±347.75	671.76±54.04	144.69±13.60	293.05±42.58
24 h	723.83±346.93	709.40±448.28	567.54±216.56	916.21±217.82
3 d	979.35±317.47	1474.92±74.26	1180.85±196.84	785.62±23.32
7 d	489.84±394.26	578.90±309.04	926.96±304.98	1907.44±607.73*

* Comparison with the control group; $P < 0.05$

Table 3 Changes of IL-4 after drug administration

IL-4	PBS control	Low dose group	Medium dose group	High dose group
8 h	21.33±16.16	19.84±0.21	9.70±3.68	10.62±0.87
24h	23.01±9.13	24.12±11.54	15.34±3.29	27.50±12.12
3 d	31.15±22.35	53.72±20.06	57.82±7.89	40.43±5.88
7 d	19.26±12.30	25.45±8.75	35.46±12.27	51.04±5.65*

* Comparison with the control group; $P < 0.05$

Table 4 Changes of IL–6 after drug administration

IL-6	PBS control	Low dose group	Medium dose group	High dose group
8 h	68.61±38.69	91.67±7.00	58.14±24.44	68.98±43.69
24 h	65.30±15.41	42.40±18.48	84.21±29.75	36.98±35.44
3 d	136.05±47.38	117.65±18.87	103.74±13.44	66.63±21.42*
7 d	49.33±24.97	57.92± 33.72	74.53±11.35	66.90±26.41

* Comparing with the control group $P<0.05$

Table 5 Changes of IL-12 after drug administration

IL-12	PBS control	Low dose group	Medium dose group	High dose group
8 h	95.43±69.25	152.59±124.51	127.22±164.05	84.97±14.64
1 d	118.65±60.42	213.70±0	74.67±0	80.93±8.85
3 d	434.00±80.17	255.19±143.80	280.25±49.23*	184.39±23.43*
7 d	140.15±105.92	61.35±11.33	237.88±75.89	175.45±26.27

* Comparison with the control group; $P<0.05$

groups than in the control group at 24 h. At the 7th day, all treatment group levels were higher than the control group levels; the high dose group in particular showed significantly higher expression than the control group(P=0.01) (Table 2, Chart 1).

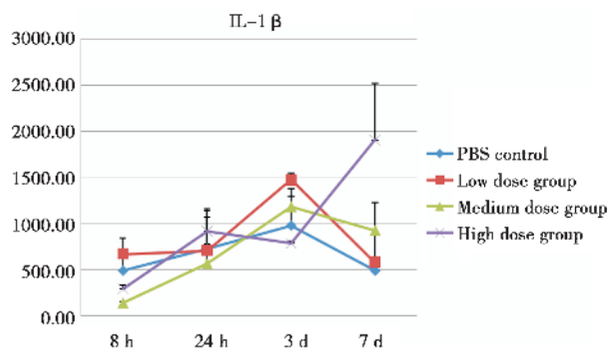

Chart 1 Changes of IL-1β after drug administration

IL-4 expression levels showed fluctuations over time, except in the high dose group. A peak appeared at 3 d after treatment and decreased afterwards, but with slight differences within each group. In particular, significantly higher expression of IL was observed in the high dose group than in the control group at day 7. A significant difference was found among the 4 groups(P=0.11) (Table 3, Chart 2).

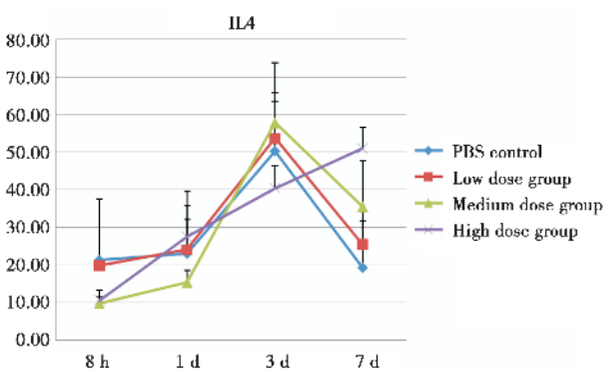

Chart 2 Changes of IL-4 after drug administration

IL-6 expression was the highest at 3 h after corneal injury and then decreased subsequently in the control group. However, the expression of IL-6 was significantly inhibited in the high dose group, especially at day 3. As shown in Table 4 and Figure 3, a significant difference was found among the 4 groups (P=0.045).

The expression level of IL12 peaked on day 3 in the control group, while, the levels in the three drug groups dropped sharply. Compared with the expres-

sion in the control group, the expression levels in the medium and high dose groups were significantly lower(P=0.047 and P=0.007 respectively) (Table 5, Chart 4).

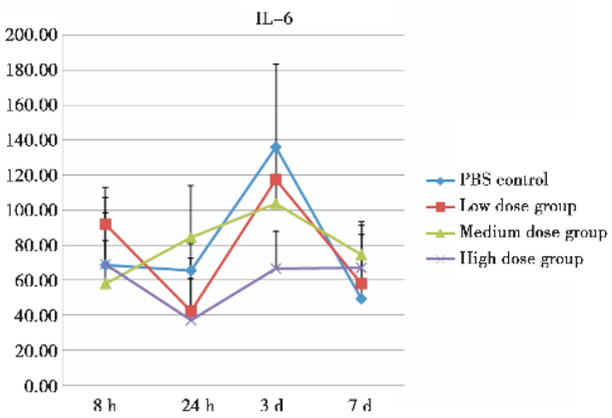

Chart 3 Changes of IL-6 after drug administration

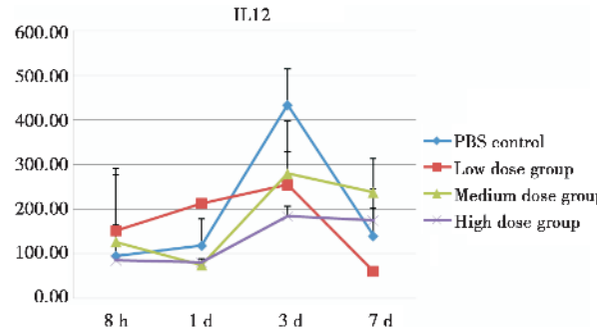

Chart 4 Changes of IL-12 after drug administration

Discussion

This study demonstrated that inflammatory cell infiltration, accompanied by changes in inflammatory cytokines, occurs in the affected corneas of HSK mouse models, which suggests that inflammatory factors are involved in HSK progression, consistent with the findings of Thomas H et al[2]. PDTC is the most common inhibitor of NF-κB and the inflammation induced by HSK can be alleviated by suppressing NF-κB, a key transcription factor regulating inflammatory factors. In addition, PDTC can inhibit the replication and transcription of viral genes[3,4]. This study shows that although IL-1β, IL-4, IL-6 and IL-12 are common downstream targets of NF-κB, they showed different change patterns in response to administration of the NF-κB inhibitor PDTC. IL-6 and IL-12 expression showed a deceasing trend, while IL-1β and IL-4 expression increased significantly, especially in the high dose group. Our results indicate that regulation by other factors must also be occurring, such as the activation of immunocytes. Wu found that PDTC was effective in the treatment of bacterial keratitis[5], whereas in our study, PDTC treatment did not result in inhibition of inflammatory cell activation, but IL-6 and IL-12 expression showed PDTC concentration dependency.

In the present study, the inflammation in the stromal HSK mouse model was initiated by HSV-1 virus; therefore, the treatment should combine anti-inflammatory and anti-viral drugs. Considering the involvement of immune factors in the pathogenesis of HSK, the role of TLR-NF-Kb receptor signaling in infectious diseases, and the function of proinflammatory factors[1], the persistence of HSV-1 antigen would be expected to significantly activate the signaling pathway of the TLR receptor, thereby causing the activation of downstream signaling of NF-κB and resulting in generation of a large amount of inflammatory cytokines when NF-κB enters the nucleus[6,7]. All of these events may act as initiation factors of immune imbalance in HSK-affected corneas. The high levels of cytokines may promote corneal injuries, destroy the functioning of T-cells, and disrupt immune protection[8], which would explain why no efficacious therapy is yet available for HSK in the clinical setting. One limitation of the present study is that the content and activity of NF-κB was not directly detected.

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