学位論文内容の要旨
（Summary of dissertation）

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学位論文題名
（Title of dissertation）

Suppressive effects of naturally-derived and novel synthetic substances on ocular inflammation.
(天然由来および新規化合物の眼炎症抑制効果)

[Background and Objectives]

Control of intraocular inflammation is important theme of modern ophthalmology. With anatomical specificity of the eye even mild inflammatory response in the confined environment of ocular globe may cause major damage and cause permanent vision loss. We used several approaches including use of antioxidant (Astaxantin), NF-κB inhibitors (IMD0354, IMD1041) and molecular chaperones (HSP70) in mouse UVB keratatis and rat endotoxin induced uveitis models.

[Methods and results]

Amelioration of ultraviolet-induced photokeratitis treated with astaxanthin eye drops in mice.

Astaxanthin (AST), 3,30-dihydroxy-b,b-carotene-4,40-dione, a carotenoid without vitamin A activity, has potential clinical applications due to its higher antioxidant activity than β-carotene and α-tocopherol. Acute ultraviolet (UV) exposure causes photokeratitis and induces various inflammatory changes in the cornea. In the present study, we examined whether topical administration of AST has therapeutic effects on UV-photokeratitis in mice.

Six- to 8-week-old C57BL/6 male mice were used. C57BL/6 male mice were administered AST in instillation form with following concentration: 1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml to right eyes, left eyes were instilled with vehicle alone. After the instillation, the mice were irradiated with UVB at the dose of 400 mJ/cm^2 under anesthesia. Eyeballs were collected 24 hours after irradiation, and stained with H&E and TUNEL. As in vitro study, NIH-3T3 cells were cultured with AST. Cytotoxicity was quantified with LDH assay.

UVB irradiation caused disruption of the corneal basement membrane and thinning of the corneal epithelium; however, the epithelium was well preserved after irradiation in AST-treated corneas. The corneal epithelium thickness was 35.75±1.7, 29.75±1.7 and 8.5±2.8 μm in mice treated with 1, 0.1 and 0.01 mg/ml of AST, respectively. The mean corneal epithelial thickness was 4.75±4.6 in untreated eyes after irradiation. Non-irradiated corneal epithelium was 38.25±2.5 μm thick. Apoptotic cells were counted as 2.75±3.7, 2.25±2.8, 19.0±3.2, and 23.0±5.3 in eyes treated with 1, 0.1, 0.01, and 0 mg/ml of AST respectively. Significantly fewer apoptotic cells were observed in AST-treated UV-irradiated mice than controls ($p<0.01$). In vitro study showed lesser cytotoxicity of NIH-3T3 cells in AST-treated cultures after UVB-irradiation. The percentages of mean cytotoxicity after irradiation were 23.0±5.3%, 59.25±5.3%, 77.75±7.6 %, and 86.75±4.3% in wells added 1, 0.1, 0.01, and 0 mg/ml of AST, respectively.

In the current study, we showed that AST has the protective effect regarding UVB irradiation in vivo and in vitro. AST might be a promising naturally-derived material protecting ocular surface from the toxicity of ultraviolet.

Amelioration of endotoxin-induced uveitis treated with IMD-0354 NF-κB inhibitor in rats.

Endotoxin-induced uveitis (EIU) is an animal model for acute ocular inflammation. There are several substances that play major roles in the development of inflammatory changes in EIU including TNF-α, Interleukin (IL)-1β IL-6 and others. This inflammatory cytokines trigger the degradation of IκB by activating IκB kinases (IKKs). Released NF-κB subsequently translocates to the nucleus, where it expresses its proinflamatory function. IMD-0354 decrease NF-κB activation by inhibition of IKK. In this study, we examined whether administration of IMD-0354 has therapeutic effects on EIU in rats.

Six-week-old male Lewis rats were used. EIU was induced by subcutaneous injections of 200 μg of LPS from *Escherichia Coli* that had been diluted in 0.1 ml of phosphate buffered saline. IMD-0354 was administered intraperitoneally 30, 10, 3 or 0 mg/kg suspended in 1.0 ml of 0.5% CMC sodium. Naïve rats were used as control. The rats were euthanized 24 hours after LPS injection, and the aqueous humor was collected, the total protein concentration in the aqueous humor samples was measured with a Qbit protein Assay kit. For cell counting, the aqueous humor was suspended in Turk stain solution, and the cells were counted using a hemocytometer under light microscopy. Some eyes in each group were fixed with PFA 4% via intracardial injection and stained with anti-NF-κB antibodies.

The total protein concentration of aqueous humor was 92.5±5.3, 101.5±11.7, 112.6±3.2 and 117.3±3.0 in rats treated with 30, 10, 3 or 0 mg/kg BW of IMD-0354, respectively. Naïve rats mean protein concentration was 21.5±4.7, it was significantly lower in IMD-0354 30 mg/kg BW ($p<0.01$) and 10 mg/kg BW ($p<0.01$) treated groups.

The number of inflammatory cells in aqueous humor was 46.4±16.8, 68.25±30.1, 128.41±54.9, and 133.3±44.0x10^4 in rats treated with 30, 10, 3 or 0 mg/kg BW of IMD-0354, respectively. There were no inflammatory cells detected in Naïve eyes.

Multiple NF-κB positive nuclei was detected in untreated eyes 249.00±27.8, There were significantly less cells detected in IMD-0354 30 mg/kg BW ($p<0.01$) 146.6±3.0 No NF-κB positive cells were detected in Naïve slides. These results suggest that IMD-0354 reduce intraocular inflammation in rat EIU by inhibition of IKK and reduced expression of NF-κB.

Induction of heat shock protein 70 ameliorates ultraviolet-induced photokeratitis in mice.

Acute ultraviolet (UV) B exposure causes photokeratitis and induces apoptosis in corneal cells. Geranylgeranylacetone (GGA) is an acyclic polyisoprenoid that induces the expression of heat shock protein (HSP)-70, a soluble intracellular protein expressed in various tissues, including the eyes. The HSPs mainly function as intracellular chaperones, protecting cells against various stress conditions. In the present study, we examined whether the induction of HSP70 has therapeutic effects on UV-photokeratitis in mice. C57BL/6 female mice were divided into four groups, GGA-treated (500 mg/kg/mouse) and UVB-exposed (400mJ/cm^2), GGA-untreated and UVB-exposed (400mJ/cm2), GGA-treated (500 mg/kg/mouse) but not UVB-exposed, and naïve controls. Eyeballs were collected 24 hours after irradiation, and corneas were stained with H&E, TUNEL, anti-HSP70 antibody, and Phospho-(serine/threonine) Akt substrate antibody. The irradiated corneal epithelium was significantly thicker in the eyes of mice treated with GGA as compared with those given vehicle alone ($P<0.01$). Significantly fewer TUNEL-positive cells were observed in the eyes of GGA-treated mice than in controls after irradiation ($P<0.01$). Corneal HSP70 levels were significantly elevated in the corneas of mice treated with GGA ($P<0.05$). Phospho-(Ser/Ther) Akt substrate expression was increased in corneas after irradiation when they were treated with GGA. GGA treatment induced HSP70 and ameliorated UV-induced corneal damage through the activation of the Akt signal.

[Conclusions]

We identified several natural and synthetic substances that reduce intraocular inflammation in UVB corneal damage model and rat EIU model. This work let to two publications on Mol Vis scientific journal. Discovered anti-inflammatory features of subscribed substances may lead to good targets to develop new drugs and supplements.