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Inhibition of crystallins-induced inflammation in rabbit eyes with five phytogenic compounds

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ABSTRACT Ocular inflammation was induced by injection of crystallins (lens protein) intracamerally and endotoxin intravitreously into rabbit and rat eyes, respectively, and was measured with fluorophotometry by quantitating the amount of fluorescein which entered into the globe. Five compounds isolated from anti-inflammatory Chinese herbs were studied for their effects on ocular inflammation. It was found that lens protein-induced inflammation was inhibited significantly by the topical instillation of pulegone (0.5%), friedelin (0.5%), and sabinene (1%), but not by dihydrojasmon or naringin at concentrations up to 1%. However, none of these compounds inhibited endotoxin-induced posterior uveitis.

KEY WORDS eye; crystallins; endotoxins; inflammation; pulegone; friedelin; sabinene; dihydrojasmon; naringin; ophthalmic solutions

Ophthalmic surgery is growing at a rapid pace because of the availability of microsurgery and the laser beam. As a result, lens protein(crystallins)-induced ocular inflammation is unavoidable and must be controlled with medication[1-4]. The immediate responses of the eye to the intraocular surgery includes hyperemia, breakdown of blood aqueous barrier, ocular hypertension, and miosis[5]. Numerous chemical mediators have been implicated for eye irritation responses, including eicosanoids[6], vasoamines[7], platelet-activating factor (PAF)[8], cytokines[9], and complements[10]. In order to avoid ocular hypertension, the production and release of chemical mediators involved in these responses have to be inhibited.

The great majority of anti-inflammatory agents used in the eye clinics are corticosteroids[11], which are known to inhibit eicosanoids and PAF production but also are reported to produce serious side effects, including acute ocular hypertension[12]. As a result, these corticosteroids are contraindicated for ocular hypertensive and glaucoma patients. Although non-steroidal anti-inflammatory agents are available (such as flurbiprofen), they are very much limited in choices[11].

Numerous Chinese herbs have been used for systemic inflammation, yet very little has been studied with isolated pure active principles on ocular inflammation[13-15]. Five compounds isolated from anti-inflammatory Chinese herbs, were studied for their effects on lens protein-induced ocular inflammation. They include: pulegone, isolated from *Glechoma longituba* (Nakai) Kupr and *Mentha rotundifolia* (L) Huds; friedelin, from *Ageratum conyzoides* (L); sabinene, from *Curcuma longa* (L); naringin, from *Citrus grandis* (L) Osbeck and *Citrus limonia* Osbeck; and dihydrojasmon, from *Jasminum sambae* (L) Ait. The effects of these agents on endotoxin-induced posterior uveitis were also studied.

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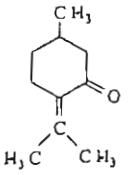
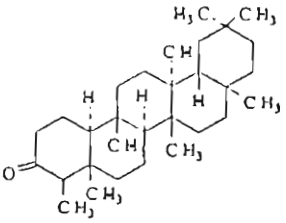


Pulegone



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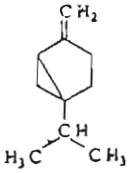
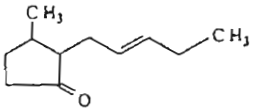
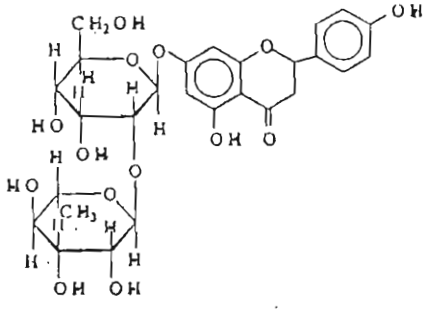


Sabinene



Dihydrojasmon



Naringin

MATERIALS AND METHODS

Materials Pulegone, friedelin, sabinene, naringin, and dihydrojasmon were purchased from Carl Roth KG (Atomergic Chem Corp, Farmingdale NY). Pulegone, sabinene, and dihydrojasmon were dissolved in a PEG 200, whereas friedelin and naringin were dissolved in mixed solvent of 60% PEG 200 and 40% Me_2SO. For lens protein-induced inflammation, 50 μl of the eyedrops were instilled into the *cul de sac*. For the endotoxin-induced posterior uveitis, the drug solutions were injected intraperitoneally three times at 0, 4, and 10 h after endotoxin injection, and the posterior uveitis were determined 12 h after the endotoxin injection. The endotoxin was purchased from Sigma (St Louis MO). Endotoxin (10 ng / 10 μl) was injected intravitreally to induce posterior uveitis in rats.

Fluorescein-labeled dextran with a molecular weight of 70 000 (FD-70) was obtained from Sigma. FD-70 (100 mg) was initially dissolved in phosphate buffer saline (Sigma) and passed through a PD-10 column (Pharmacia). This solution was then diluted with heparinized normal saline to make a final concentration of FD-70 10 mg· ml^{-1} and heparin 10 U· ml^{-1}.

Lens protein was prepared and protein concentration was determined according to procedures described by Miyano, *et al*[1]. The quantity of lens protein in the lens protein preparation was 29.67 mg· ml^{-1}.

Lens protein-induced inflammation in rabbit eyes New Zealand albino rabbits of either sex, weighing 2.0-3.0 kg, were used. The rabbits were initially anesthetized with ketamine 40 mg· kg^{-1} and xylazine 5 mg· kg^{-1} given intramuscularly. Half of the above dosage was given hourly for the remainder of the experiment. Fifteen minutes after the anesthesia, 50 μl of the solvent were instilled into the right eye. Fifty microliters of the drug solution were instilled into the left eye. One hour after the application of the drug and the vehicle, 25 μl of the lens protein were injected into the anterior chamber with a 30 1 / 2 gauge needle. Extreme care was taken to avoid traumatizing the iris. Fifteen minutes after the injection of the lens protein, FD-70 (1.4 mg· kg^{-1}) was injected via a marginal ear vein.

Scanning of the eyes were done by the use of a fluorophotometer (Fluorotron Master, Coherent Corp, Palo Alto CA). Measurements were done at 0, 30, 60, 90, 120, 180, 240, 300, and 360 minute intervals. The measurements are expressed in FD-70 μg· ml^{-1} in the eye.

Endotoxin-induced posterior uveitis in rat eyes Sprague-Dawley rats, weighing 250-350 g, were anesthetized with ketamine 25 mg· kg^{-1} and xylazine 5 mg· kg^{-1} intramuscularly. Endotoxin (10 ng / 10 μl) was injected intravitreally, and the rats were allowed to recover from the anesthesia. The

Tab 1. Fluorescein-labeled dextran (pg · ml^{-1}) in crystallins-induced inflammation in rabbit eyes treated by eyedrops of 5 phytogenic compounds and ip prednisolone 20 mg · kg^{-1} thrice. $\bar{x} \pm s$. $^{*}P > 0.05$. $^{**}P < 0.05$ $^{***}P < 0.01$ *vs* **Control.**

Time/h	Control	Treated
	(n=6)	0.5% Pulegone (n=6)
0.5	320± 149	138± 110**
1	622± 286	196± 118***
1.5	747± 239	303± 157***
2	731± 262	373± 176**
3	708± 214	334± 189***
4	583± 207	277± 138**
5	471± 215	181± 212**
6	346± 133	100± 86***
	(n=5)	1.0% Pulegone (n=5)
0.5	674± 276	90± 97***
1	885± 280	241± 236***
1.5	864± 266	248± 169***
2	872± 188	234± 140***
3	734± 130	163± 73***
4	664± 298	90± 39***
5	386± 162	50± 27***
6	258± 107	27± 18***
	(n=6)	0.5% Friedelin (n=6)
0.5	255± 173	49± 40*
1	660± 438	223± 98*
1.5	807± 377	302± 197**
2	840± 407	361± 186**
3	575± 265	303± 179***
4	338± 114	235± 119**
5	273± 95	146± 86***
6	196± 63	101± 54***
	(n=6)	1.0% Friedelin (n=6)
0.5	345± 173	88± 68***
1	913± 263	287± 157***
1.5	1 090± 249	299± 144***
2	1 082± 194	289± 92***
3	785± 307	266± 76***
4	556± 194	151± 80***
5	527± 167	98± 79***
6	422± 133	69± 65***
	(n=5)	1.0% Sabinene (n=5)
0.5	152± 78	133± 101*
1	1 017± 246	442± 377**
1.5	956± 178	470± 313**
2	960± 205	479± 257**
3	693± 170	293± 197***
4	510± 200	146± 120***
5	406± 180	73± 67***
6	314± 209	58± 54***
	(n=6)	2.0% Sabinene (n=6)
0.5	550± 521	68± 80**
1	991± 597	177± 100***
1.5	1 144± 366	219± 92**
2	1 066± 377	392± 174***
3	945± 245	387± 109***
4	732± 143	379± 120***
5	420± 173	218± 54**
6	351± 127	143± 39***
	(n=6)	1.0% Naringin (n=6)
0.5	83± 53	82± 55*
1	459± 324	430± 449*
1.5	675± 395	431± 309*
2	669± 243	450± 257*
3	661± 54	394± 239**
4	503± 121	174± 123***
5	306± 78	129± 99***
6	174± 54	74± 61**
	(n=8)	1.0% Dihydrojasmon (n=8)
0.5	216± 261	163± 120*
1	445± 400	267± 257*
1.5	445± 317	334± 238*
2	527± 276	351± 244*
3	488± 177	291± 179**
4	352± 188	192± 125*
5	270± 144	139± 107***
6	194± 75	86± 60***
	(n=4)	Prednisolone (n=6)
0.5	81± 17	22± 8***
1	243± 87	41± 26***
1.5	309± 140	60± 32***
2	430± 170	80± 43***
3	495± 295	111± 52**
4	533± 234	129± 54***
5	567± 222	149± 63***
6	581± 279	154± 55***

compounds to be studied and prednisolone at various doses were injected intraperitoneally at 0, 4, and 10 h after the endotoxin injection. The uveitis was measured with a Fluorotron (fluorophotometer) at 12 h after the endotoxin injection.

Statistical analysis Each value was expressed as mean± standard deviation. All data were analyzed with *t* test for two values and analysis of variance for more than two values.

RESULTS

When 50 μl of 0.5% pulegone were instilled into the *cul de sac* of the rabbit eyes, the lens protein-induced inflammation was markedly inhibited (58% inhibition based on area under curves). Both early phase and late phase of the inflammation were inhibited by pulegone. Further inhibition was observed with 1.0% pulegone (80% inhibition based on area under curve), showing a good dose-response relationship between the 2 dose levels. Similar results were obtained with friedelin eyedrops. The lens protein-induced inflammation in the rabbit eyes was inhibited 50% and 73%, respectively, based on the area under curve calculation, by 0.5% and 1.0% friedelin eyedrops. Again, both early and late phases of the inflammation were inhibited effectively by friedelin. In the case of sabinene, it took higher doses (1.0% and 2.0% eyedrops) to inhibit the inflammation 61% and 65%, respectively. Naringin and dihydrojasmon produced only marginal inhibition of the lens protein-induced inflammation at concentrations up to 1.0%. Prednisolone (10-20 $mg \cdot kg^{-1}$ ip 3 times) effectively inhibited endotoxin-induced posterior uveitis. (Tab 1)

However, none of the five compounds tested showed any inhibition of the posterior uveitis at doses up to 10 $mg \cdot kg^{-1}$ ip 3 times.

DISCUSSION

It is exciting to note that pulegone, friedelin and sabinene markedly inhibited lens protein-induced ocular inflammation both in the early and late phases, similar to corticosteroids, although they are not corticosteroids. Since these compounds did not inhibit endotoxin-induced posterior uveitis and since endotoxin is known to induce inflammation through release of cytokines, such as interleukin-1, interleukin-6, and tumor necrosis factor, their effects are probably related to the inhibition of eicosanoids and PAF production rather than to that of cytokines, vasoamines or complements.

Since the Chinese herbs containing these natural non-steroidal anti-inflammatory agents have been used for centuries in Asia without producing serious side effects in humans, it is hoped that they can be used for ocular inflammation, similar to corticosteroids, but without producing as many side effects as the corticosteroids.

REFERENCES

1 Miyano K, Chiou GCY. Pharmacological prevention of ocular inflammation induced by lens proteins. *Ophthalmic Res* 1984; **16** : 256-63.

2 Chang MS, Chiou GCY. Prevention of lens protein induced ocular inflammation with cyclooxygenase and lipoxygenase inhibitors. *J Ocular Pharmacol* 1989; **5** : 353-60.

3 Chiou GCY, Chang MS. Ocular inflammation induced by lens protein and its prevention with new agents. In: Braquet P, editor. *New trends in lipid mediators research*; vol 5: *Lipid mediators in eye inflammation*. Basel: Karger, 1990: 94-109.

4 Millichamp NJ, Dziezyc J, Rohde BH, Chiou GCY, Smith WB. Acute effects of anti-inflammatory drugs on neodymium: yttrium aluminum garnet laser-induced uveitis in dogs. *Am J Vet Res* 1991; **52** : 1279-84.

5 Unger WG. Mediation of the ocular response to injury and irritation: peptides versus prostaglandins. *Prog Clin Biol Res* 1989; **312** : 293-328.

6 Bhattacherjee P. The role of arachidonate metabolites in ocular inflammation. *Prog Clin Biol Res* 1989; **312** : 211-27.

7 Butler JM, Hammond BR. The effects of sensory denervation on the responses of the rabbit eye to prostaglandin E_2, bradykinin and substance P. *Br J*

Pharmacol 1980; **69** : 495–501.
8 Rubin RM, Samples JR, Rosenbaum JT. Prostaglandin-independent inhibition of ocular vascular permeability by a platelet-derived factor antagonist. *Arch Ophthalmol* 1988; **106** : 1116–20.
9 Melli M, Parente L. *Cytokines and lipocortins in inflammation and differentiation.* New York: Wiley-Liss, 1990 : 138–444.
10 Roitt I, Brostoff J, Male DK. *Immunology.* St Louis: Mosby, 1985 : 7.1–7.14.
11 *Physicians' Desk Reference for Ophthalmology.* Oradell: Med Economics Co, 1990 : 9.
12 Leopold IH, Murray D. Noncorticosteroidal anti-inflammatory agents in ophthalmology. *Ophthamology* 1979; **86** : 142–55.
13 Jiansu New Medical College. *Chinese traditional medicine encyclopedia.* Shanghai: Shanghai People's Pub Co, 1975 (Suppl) : 639–41.
14 Chiou GCY, Shen ZF, Zhao F. Treatment and prevention of ocular inflammation with Chinese herbs. *Int . Oriental Med* : 1991; **16** : 144–50.
15 Chang MS, Xiao JG, Chiou GCY. Prevention of ocular inflammation by matrine, prednisolone and cyclooxygenase and lipoxygenase inhibitors. *Acta Pharmacol Sin* 1991; **12** : 121–5.

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Effects of menadione on 1,2-dimethylhydrazine-induced mouse colon adenocarcinoma

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ABSTRACT The effects of menadione (Vit K_3) administered at 20 or 40 mg·kg^{-1} ig 3 times a week for both 24 and 28 wk on 1,2-dimethylhydrazine (DMH)-induced mouse colon adenocarcinomas were investigated. At the 24th wk, the number of colon tumors in Vit K_3 20 or 40 mg·kg^{-1} group (0.3± 0.5 and 0.5± 0.8, respectively) was less than that of DMH controls (2.1± 2.5, $P<0.05$), but the difference in incidence of colon tumors in these 3 groups was not significant ($P>0.05$). After 28 wk, the tumor incidence of both Vit K_3 groups (each 8 of 13) was lower than that of DMH controls (13 of 13, $P<0.05$); the number of colon tumors of Vit K_3 40 mg·kg^{-1} group (1.3± 1.3, $P<0.05$) was decreased, whereas the Vit K_3 20 mg·kg^{-1} group (3.0± 5.1, $P>0.05$) was not different from the DMH controls (7.3± 9.3). Determination of the nuclear DNA content of cells from DMH-induced mouse colon mucosa (24 wk) indicated that Vit K_3 20 or 40 mg·kg^{-1} group showed lower DNA content (1.92 ± 0.12 C and 1.91± 0.10 C, respectively) decreased values of percent-over-3C and -4C and narrow distribution range. Besides, the colon mucosa of DMH-treated mice (28 wk) showed higher superoxide dismutase (SOD) activity (70± 28 U/mg protein, $P<0.05$) than the normal controls (30± 20 U/mg protein). Vit K_3 40 mg·kg^{-1} reduced the elevated SOD activity markedly (44± 23 U/mg protein, $P<0.05$).

KEY WORDS vitamin K; methylhydrazines; colonic neoplasms; DNA; superoxide dismutase; mucous membrane

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Recently, a great deal of interest has been focused on the cancer prevention and treatment by vitamins. Investigators discovered that the menadione (Vit K_3) had antitumor activity *in vitro* comparable to the toxic anthracyclic quinones doxorubicin and daunorubicin[1]. Vit K_3 enhanced the antineoplastic activity of 5-fluorouracil in Friend murine erythroleukemia cells *in vitro*[2], and of methotrexate in tumor-bearing animals[3]. Combined administration of vitamin C, K_3,