

Effect of Propolis on Endotoxin-Induced Uveitis in Rabbits

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Purpose: To test the anti-inflammatory effect of propolis, a natural bee-produced compound, and compare it with corticosteroids for the treatment of endotoxin-induced uveitis (EIU).

Methods: EIU was produced in all rabbits by unilateral intravitreal injection of 2,000 ng *Salmonella typhimurium* endotoxin. The animals were then divided randomly into three groups as follows: group A received no treatment (control); group B received methylprednisolone (5 mg/0.1 mL) (positive control); and group C received propolis (5 mg/0.16 mL) by anterior sub-Tenon injection at the time of uveitis induction and at 4 and 8 hours after induction. Inflammation was evaluated by clinical manifestations and by measuring the protein concentration and inflammatory cell content of the aqueous humor.

Results: The clinical grade, cell count, and protein levels in the aqueous humor were: control group (6.0 ± 0.8 , $2,519 \pm 470$ cells/ μ L, 32.9 ± 2.4 mg/mL); methylprednisolone group (1.8 ± 0.7 , 572 ± 137 cells/ μ L, 15.2 ± 1.8 mg/mL); and propolis group (2.3 ± 0.5 , 503 ± 124 cells/ μ L, 13.8 ± 1.5 mg/mL). Statistically significant differences were recorded in the treatment groups when compared to the control group ($P < .001$). The effects of methylprednisolone and propolis on EIU were similar ($P > .05$).

Conclusions: Propolis showed significant anti-inflammatory effects on EIU in rabbits. The mechanism of its action warrants further investigation. **Jpn J Ophthalmol 1999;43:285–289**
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Key Words: Anti-inflammatory effect, endotoxin-induced uveitis, propolis, rabbit, *Salmonella typhimurium*.

Introduction

Endotoxin-induced uveitis (EIU) serves as a model for certain types of human ocular inflammation, collectively termed uveitis, that appear in Reiter's syndrome, dysentery syndromes, Crohn's disease, ulcerative colitis, and Behçet's disease.¹ The inflammatory reaction is maximal 24 hours after the endotoxin injection and subsides after 5–7 days.^{2,3} New Zealand rabbits and different strains of rats are usually used in EIU experiments. *Salmonella typh-*

imurium endotoxin, a lipopolysaccharide (LPS), is commonly used for the uveitis induction. The endotoxin is usually injected into the footpad or into the vitreous body of the animal.

The ocular inflammation in EIU is characterized by an alteration in vascular permeability, with leakage of protein and inflammatory cells into the iris, ciliary body, and both chambers.^{1–4} Arachidonic acid metabolites such as prostaglandin E₂ (PGE₂), thromboxane B₂ (TXB₂), and leukotriene B₄ (LTB₄) have been implicated as important mediators in EIU.^{3,4}

Corticosteroids are the most commonly used drugs in the treatment of uveitis. Although corticosteroids are capable of reducing the inflammation from EIU in animals and from anterior uveitis in humans, adverse side effects are not uncommon with prolonged use of corticosteroids. Thus, agents with

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potent anti-inflammatory effects without associated side effects need to be investigated.⁵

Propolis is a natural product collected by bees from tree buds. It has long been used in folk medicine. Previous studies have documented that propolis had strong anti-inflammatory,⁶⁻¹⁰ antimicrobial,⁶ antioxidant,¹¹⁻¹⁴ immunostimulatory, and carcinostatic activities.¹⁵ Propolis causes inhibition of leukotriene production and prostaglandin formation and has an important anti-inflammatory effect.^{6,7} It was shown that propolis exhibited anti-inflammatory effects comparable to diclofenac⁷ and hydrocortisone⁶ in certain experimental models.

In this study, we tested the anti-inflammatory effect of propolis on an EIU model in rabbits.

Materials and Methods

Animals

Twenty-six New Zealand white rabbits, each weighing 2–2.5 kg, were used. Animals were fed food and water ad libitum. The animals were treated humanely according to the guidelines of the Association for Research in Vision and Ophthalmology.

Drugs

Drugs were purchased from Sigma (St. Louis, MO, USA): 2,000 ng of *S. typhimurium* LPS (L6511; Sigma) was diluted in 10 µL of sterile pyrogen-free saline; a 3% ethanolic extract of propolis (P8904; Sigma) (EEP, pH 7.3), was prepared in phosphate-buffered saline (PBS) so that 5 mg of propolis was contained in 0.16 mL. Methylprednisolone (Prednol-L; M. Nevzat, Istanbul, Turkey) (5 mg/0.1 mL) was used as the corticosteroid.

Induction and Monitoring of EIU

The 2,000 ng of LPS diluted in 10 µL of sterile saline solution was injected into the vitreous of the right eyes of all animals using a scalp vein needle connected to a Hamilton syringe. Intravitreal injections were made through the pars plana after the animals had been sedated with ketamine (20 mg/kg im) and topical oxybuprocaine.

Clinical Score of Anterior Uveitis

Slit-lamp examinations were performed by two masked investigators. The intensity of the intraocular inflammation was graded using a clinical scoring system described previously (Table 1).¹⁶

To observe the time of the maximal inflammatory response to LPS, both eyes of two rabbits were in-

Table 1. Scoring System for Clinical Evaluation of Uveitis¹⁶

Clinical Signs	Grade of Uveitis (Score)
Iris hyperemia	
Absent	0
Mild	1
Moderate	2
Severe	3
Pupil	
Normal	0
After miosis	1
Exudate in anterior chamber	
Absent	0
Small	1
Large	2
Hypopyon	
Absent	0
Present	1
Maximum possible score	7

jected with LPS intravitreally. All eyes presented a fibrinous exudate and dense flare in the anterior chamber 20 hours after the injection, and the inflammation peaked at 24 hours (mean clinical grade, 5.9 ± 0.7). The inflammatory reaction subsided by day 5.

Treatment

Animals were randomly divided into three groups (eight per group). Group A rabbits received no treatment and served as controls. Group B received methylprednisolone in the right eyes, which served as positive controls. Group C received propolis in the right eyes. Both methylprednisolone and propolis were administered by anterior sub-Tenon injection at the time of uveitis induction and at 4 and 8 hours after the induction. Five milligrams of each substance were administered in each injection. The left eyes of group A were used as negative controls ($n = 8$) and were injected with sterile saline solution intravitreally (10 µL) at the time of EIU induction and by sub-Tenon route (1 mL) during therapy. The left eyes of group B and group C served as the intact control eyes ($n = 16$).

Sampling

Twenty-four hours after the intravitreal injection of LPS, the animals were sacrificed by an overdose of intravenous sodium pentobarbital. The aqueous humor was sampled immediately by paracentesis (with 30-gauge needles and tuberculin syringes). One µL of aqueous aspirate was placed on a glass slide,

air-dried, and then stained by Wright's method. The total number of inflammatory cells in each 1 μ L sample was then counted under a microscope. The remainder of the aqueous specimen was reserved for analysis of protein content.

Protein Levels

Protein concentrations were determined by the method of Lowry et al.¹⁷

Statistical Analysis

Data are reported as mean \pm standard deviation (SD). Between-group comparisons were made using the Kruskal-Wallis and Mann-Whitney *U* tests. A *P* value less than .05 was considered significant.

Results

Clinical Observations

All group A eyes injected with LPS (8 out of 8) developed an ocular inflammation that peaked at 24 hours with a mean clinical grade of 6.0 ± 0.8 (Table 2). No inflammatory signs were observed in the intact contralateral eyes; only a mild inflammatory response, characterized by conjunctival hyperemia, was found in those eyes that had received intravitreal and sub-Tenon injections of sterile saline (negative control, group A).

Treatment with methylprednisolone (mean clinical grade, 1.8 ± 0.7) and propolis (mean clinical grade, 2.3 ± 0.5) reduced the clinical score significantly when compared to the data for group A rabbits (controls) (*P* < .001). The effects of methylprednisolone and propolis were similar (*P* > .05).

Leukocytes were not found in the intact eyes of groups B and C. The cell counts in the aqueous humor were significantly higher in the control group (group A) ($2,519 \pm 470$ cells/ μ L) than in methylprednisolone- (572 ± 137 cells/ μ L, group B) and propolis-treated (503 ± 124 cells/ μ L, group C) groups.

Protein Concentration in the Aqueous Humor

The mean protein content in the aqueous humor of the LPS-injected control eyes was 32.9 ± 2.4 mg/mL, which was significantly higher than the 1.4 ± 0.5 mg/mL in the negative control eyes. Traces of proteins (0.23 ± 0.1 mg/mL) were detected in the aqueous humor of the intact control eyes. The aqueous humor protein levels were significantly higher in the control group (32.9 ± 2.4 mg/mL) than in the methylprednisolone (15.2 ± 1.8 mg/mL) and the propolis (13.8 ± 1.5 mg/mL) groups (*P* < .001). This indicated that the breakdown of the blood–aqueous barrier was prevented by methylprednisolone and propolis. The effects of prednisolone and propolis were similar (*P* > .05).

Discussion

Methylprednisolone and propolis were found to significantly inhibit the development of EIU in rabbits. This model of EIU is well established¹⁻⁴ and has been considered a good model of human anterior uveitis. EIU has been used for the study of the human iridocyclitis that appears in Reiter's syndrome, dysentery syndromes, Crohn's disease, ulcerative colitis, and Behçet's disease.¹ Acute anterior uveitis in humans is of short duration and is characterized by the presence of neutrophils and protein exudates in the anterior chamber. These features are very similar to those seen in EIU. EIU also provides a useful model for the investigation of new anti-inflammatory agents.^{5,18,19}

A single intravitreal injection of LPS induces acute uveitis in rabbits.²⁰⁻²² Because LPS induces a variety of inflammatory signs and events, a wide array of proinflammatory mediators, including cytokines,^{16,21,23} prostaglandins and leukotrienes,^{3,4} platelet-activating factors,²⁴ oxygen free radicals,²⁵ and others, have been implicated in the pathogenesis of EIU.²² The exact mechanisms leading to EIU are not

Table 2. Summary of Data on EIU Parameters

Group	Aqueous		
	Clinical Grade	Cells/ μ L	Protein (mg/mL)
1. Control (LPS) (n = 8)	6.0 ± 0.8	$2,519 \pm 470$	32.9 ± 2.4
2. LPS + M. Prednisolone (n = 8)	$1.8 \pm 0.7^*$	$572 \pm 137^*$	$15.2 \pm 1.8^*$
3. LPS + Propolis (n = 8)	$2.3 \pm 0.5^*$	$503 \pm 124^*$	$13.8 \pm 1.5^*$
4. Negative control (n = 8)	0.6 ± 0.5	6 ± 3	1.4 ± 0.5
5. Intact control (n = 16)	0	0	0.2 ± 0.1

Values are given as mean \pm SD. n = number of eyes in each group.

**P* < .001, with respect to control.

well known. It has been shown that after an inflammatory stimulus *in vivo*, ocular tissues can release both lipoxygenase and cyclooxygenase products of arachidonate metabolism such as LTB₄, PGE₂, and TXB₂, which have been implicated as important mediators in EIU.^{3,4}

The ocular inflammatory response includes both the early breakdown of the blood–aqueous barrier, resulting in protein extravasation into the aqueous humor, and the influx of polymorphonuclear leukocytes into the iris, ciliary body, and both chambers.^{1–4}

Glucocorticosteroids are very effective drugs and are widely used for the treatment of ocular inflammation. The type of steroid preparation and the method of administration can lead to marked differences in the penetration and efficacy in EIU eyes.^{26,27} Topical prednisolone phosphate (1%) and acetate (1%) significantly reduced endotoxin uveitis in the rabbit.^{28,29} We used methylprednisolone by the anterior sub-Tenon route, as was done for the propolis administration.

In this model, propolis showed an anti-inflammatory effect comparable to methylprednisolone. Propolis is a natural hive product from the honey bee (bee glue). The chemical composition of propolis appears to be very complex; to date at least 156 propolis constituents have been identified.^{30–33} It possesses versatile biologic activities including antimicrobial, anti-inflammatory, regenerative, antioxidant, and cytostatic effects.^{30–32} The wide spectrum of propolis activity is attributed to the large number of flavonoid compounds and caffeic acid phenethyl ester (CAPE) it contains.^{10–15}

In previous studies, propolis extracts were shown to possess significant anti-inflammatory properties, comparable to hydrocortisone in treating formaldehyde-induced arthritis (chronic inflammation) and PGE₂-induced paw edema (acute inflammation), and comparable to diclofenac in treating adjuvant-induced arthritis (chronic inflammation) and carrageenan-induced paw edema (acute inflammation).^{6,7}

The observed anti-inflammatory effect of propolis could be attributed to its contents of flavonoids, phenolic acid and caffeic acid.^{7,10} Flavonoids were reported to inhibit the activity of enzymes involved in the conversion of membrane polyunsaturated fatty acids such as phospholipase A₂, cyclooxygenase, and lipoxygenase, to inhibit the release of the lysosomal enzymes from rabbit polymorphonuclear leukocytes, and to scavenge free radicals.⁷ Aqueous extracts of propolis were found to have an inhibitory effect on enzyme dihydrofolate reductase similar to the well-known nonsteroidal anti-inflammatory drugs.⁸ This property may explain part of its anti-inflammatory action.

CAPE, which is an active component of propolis extract, was found to inhibit 5-lipoxygenase in micromolar concentrations, and to block the production of reactive oxygen species in human neutrophils and the xanthine/xanthine oxidase system. It was also believed to contribute to the anti-inflammatory activity of propolis by being both a lipoxygenase inhibitor and an antioxidant.¹²

The levels of free radicals, such as nitric oxide (NO) and malondialdehyde, increase in EIU, and antioxidant agents were found to be effective in EIU.^{22,34} Treatment with the 5-lipoxygenase inhibitor attenuated the intensity of cellular infiltration and protein extravasation in EIU.³⁵ Recently, it was shown that the concomitant inhibition of the lipoxygenase pathway, as well as the reduction in oxygen free radicals, could improve the anti-inflammatory effect of NO synthesis inhibitors (N^G-nitro-L-arginine methyl ester) during the early phase of EIU.²² Propolis is considered to be a regulator of free radical concentrations in various pathological conditions.^{11–14} CAPE was found to inhibit 5-lipoxygenase.¹² The antioxidative and anti-inflammatory properties of propolis might have a role in inhibiting the development of EIU.

Our data confirm the anti-inflammatory effect of propolis in the rabbit model of EIU. Given these results, propolis might be a useful anti-inflammatory agent in selected ocular inflammatory conditions. The mechanisms of action of propolis warrant further investigation.

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