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The Anti–inflammatory Effects of N^G–Nitro L–Arginine (L-NAME) and Steroid in Concanavalin A–Induced Uveitis

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Abstract: The objective of this study was to compare the anti-inflammatory effects of NG -nitro L-arginine (L-NAME) and corticosteroid in Concanavalin A-induced uveitis in rats. Sixteen male Wistar rats were used. After general anesthesia, intravitreal 0.1 ml Concanavalin A (100μg/ml) was injected into the left eyes of the rats. The animals were divided into 3 groups: group 1 (6 animals) received intraperitoneal 0.2 ml L-NAME (200 mg/kg) 1 hour before, 1 day and 3 days after Concanavalin A injection, group 2 (6 animals) received tropical 1% prednisolone acetate four times a day for 3 weeks, group 3 (4 animals) received an intraperitoneal injection of 0.2 ml balanced salt solution (BSS) 1 hour before, 1 day and 3 days after Concanavalin A injection, as a control group. Anterior and posterior inflammations were observed with a slit lamp. Three weeks after the last injection, all eyes treated with L-NAME and topical steroid showed significantly reduced anterior chamber inflammation, while eyes which received BSS showed moderate to severe inflammation in both anterior and posterior segments. Both drugs showed no real effect on the vitreous humor at the end of follow up period. In conclusion, our feeling is that tropical steroid appears to still be the mainstay therapy for the treatment of anterior uveitis, but nitric oxide synthase (NOS) inhibition might be an alternative to steroids as a second line drug, at least whenever there are any adverse reactions or contraindications to corticosteroid drugs. L-NAME might also be effective on vitreous inflammation with new application methods and concentrations.

Key Words: Concanavalin A, L-NAME, Steroid, Uveitis.

Introduction

Uveitis is a chronic inflammatory condition of the eye involving both anterior and posterior segment. It remains a major cause of significant visual loss worldwide(1). One of the main sight-threatening complications is macular edema which often causes deep loss of central vision and may or may not respond to conventional therapies.

Any immunologic or inflammatory stimuli induce the production of nitric oxide (NO) by the expression of the inducible isoform of the nitric oxide synthase (NOS) (2). It is well known that NO is involved in different kinds of inflammatory conditions such as arthritis, colitis and nephritis (3–5). Concanavalin A is a nonspecific inflammatory agent, which has been used in many previous experimental studies to induce uveitis (6–8). Current management of uveitis consists of suppressing the immune system to reduce the inflammatory response, using either local or systemic drugs (9). Corticosteroids still remain the major choice for uveitis treatment, which also reduce the induction of NOS in many organs (10–11). Another drug used in this study was L-NAME, an inhibitor of NOS, reduces the inflammation in the eye (12).

We compared the anti-inflammatory effects of L-NAME and corticosteroid as a first in Concanavalin A-induced inflammation.

Materials and Methods

Sixteen male Wistar rats (6 to 8 weeks of age, 150 to 200 g) were used. The animals were handled and cared for according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The rats were

anesthetized with intraperitoneal injection of 50 mg/kg ketamine HCI and 10 mg/kg xylazine. Corneal anesthesia was achieved with topical 0.4% oxbuprocaine HCL.

All injections of Concanavalin A (100 mg/ml, Sigma, St. Louis, MO) were only in the left eyes of the rats, using a 30-gauge needle. After that the rats were divided into 3 groups. Group 1 was treated with a 0.2 ml intraperitoneal injection of L-NAME (200mg/kg, Sigma) 1 hour before, 1 day and 3 days after Concanavalin A injection. Group 2 was treated with topical 1% prednisolone acetate (Pred Forte, Allergan) four times a day for 3 weeks. Group 3 received an intraperitoneal injection of equal volume of BSS at the same times with L-NAME injections. Postoperatively, all ayes received 1% cyclopentolate 2 times daily for 2 weeks to maintain dilation. All eyes were examined with slit lamp biomicroscopy findings were graded on a scale from 0 to 4 with 0=none, 1=trace, 2=mild, 3=moderate, and 4=severe (Table 1). The Wilcoxon Rank test was used for intergroup comparisons.

Table 1. Inflammation Grading Scale.

Cells	Grade	Description
None	0	No cells seen per high power field
Trace	1	1-9 cells seen per high power field
Mild	2	10-25 cells seen per high power field
Moderate	3	26-50 cells seen per high power field
Severe	4	More than 50 cells seen per high power field

Results

Our results are shown in Tables 2 and 3. On day 3, all eyes in group 1 and 2 showed none to trace cells both in the anterior chamber and in the vitreous humor. Group 3 eyes showed trace cells, and none to trace cells in the anterior and posterior segments, respectively.

Anterior segment inflammation gradually subsided after between 7 and 15 days in groups 1 and 2, while posterior segment inflammation increased with mild cells. There was evidence of inflammation in all BSS-treated eyes with mild cells in the anterior chamber and moderate inflammation in the vitreous humor on these days.

The anterior segments were normal in steroid and L-NAME-treated groups on the 18th and 21st day after Concanavalin A injection, respectively. However, on the 21st day, either anterior or posterior segment inflammations were at moderate levels in the control group. The posterior segment iflammation peaked on the 21st day, then started to gradually decrease from day 21 to 45 in all groups. No significant differences were observed betweet groups 1 and 2 in either segment at any time ($p>0.05$). The differences were statistically significant between the-treatment groups and the control group in the anterior segment inflammation ($p=0.001$ to 0.04). However, the difference was statistically significant only on days 21, 35, and 45 in the posterior segment ($p=0.02$ to 0.04). After 45 days, it was difficult to evaluate vitreous inflammation due to the development of cataracts.

Discussion

The spectrum of uveitis constitutes one of the major causes of blindness. Uveitis is an iflammation of the iris, ciliary body or choroid. It is not a specific disease, and it can be caused by a multitude of conditions, including antigen specific immune mediated inflammation, infection, trauma, and surgery (13). The inflammatory response in the eye consists of miosis, conjunctival hyperemia and breakdown of the blood-aqueous barrier with subsequent leakage of protein into the aqueous humor.

Table 2. Anterior Chamber Ocular Inflammation at Different Time Intervals.

Day	Group 1	Group 2	Control	P Value (Between groups 1,2 and control)
3	0.84 ± 0.17	0.75 ± 0.14	1.81 ± 0.32	0.040
7	0.66 ± 0.15	0.59 ± 0.21	2.06 ± 0.13	0.028
15	0.31 ± 0.21	0.25 ± 0.19	2.44 ± 0.28	0.005
18	0.16 ± 0.18	0.0	2.61 ± 0.17	0.002
21	0.0	0.0	3.04 ± 0.24	0.001

Values are mean ± SE. Blomicroscopy findings are graded on a scale from 0 to 4; 0=none, 1= trace, 2=mild, 3=moderate,, and 4=severe.

The animal model of experimental Concanavalin A uveitis has been widely used for immunogenic studies and has been shown to induce uveitis after intravitreal injection(6-8). It is a nonspecific inflammatory agent and mitogen for T cells and some B cells.

New therapeutic approaches and drugs such as cyclosporin A, azothioprine, cyclophosphamide, indomethacin are being used and hyperemia also assessed as second line therapies, especially when steroids alone are not effective or too high a dose in required to achieve the desired effects (14,15). Corticosteroids have both immunosuppressive and anti-inflammatory actions (16,17). Part of the these actions is due to inhibition of the induction of the NOS (18). NO is generated from L-arginine by the enzyme NOS, which is inhibited effectively either in vitro or in vivo, by analogues of L-NAME (19). Two types of NOS have been identified, one constitutive and Ca^{2+}-dependent and the other inducible and Ca^{2+}-independent (19). The inducible form is found in many types of cells, such as macrophages, neutrophils, endothelial cells, vascular smooth muscles, and retinal pigment epithelial cells (20,21). NOS activity has been measured in the anterior uvea of the rabbit (22). Intraperitoneal injections of L-NAME were shown to reduce the formation of nitric oxide and prevent the clinical, histological signs of uveitis (12). Macrophages play a crucial role in the inflammatory process and express high affinity receptors for corticosteroids (23). The inhibition of induction of the inducible NOS in macrophages by corticosteroids is possibly mediated via interaction with specific receptors.

In summary, we compared L-NAME and corticosteroid for the first time in experimental Concanavalin A-induced uveitis. Our data shows that intraperitoneal L-NAME is as effective as topical steroid in reducing anterior chamber inflammation, but needs further investigation to find more suitable application routes such as topical. In the posterior segment inflammation, neither drug appeared to be effective. On the other hand, our feeling is that intravitreal L-NAME application at different concentrations might be more effective in the treatment of vitreous inflammation. Finally, this study demonstrates the therapeutic potential of NOS inhibition for the treatment of anterior uveitis, especially whenever any adverse reactions to corticosteroid eye drops are suspected or occur.

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Table 3. Posterior Chamber Ocular Inflammation at Different Time Intervals.

Day	Group 1	Group 2	Control	P Value (Between groups 1,2 and control)
3	0.18 ± 0.16	0.16 ± 0.14	0.71 ± 0.20	0.083
7	1.83 ± 0.30	1.94 ± 0.25	2.74 ± 0.21	0.064
15	2.32 ± 0.18	2.35 ± 0.22	3.08 ± 0.16	0.072
18	2.41 ± 0.33	2.39 ± 0.24	3.14 ± 0.16	0.072
21	2.60 ± 0.29	2.67 ± 0.19	3.82 ± 0.33	0.033
35	2.13 ± 0.19	2.20 ± 0.23	3.52 ± 0.15	0.023
45	1.48 ± 0.18	1.65 ± 0.31	2.60 ± 0.17	0.046

Values are mean ± SE. Biomicroscopy findings are graded on a scale from 0 to 4; 0=none, 1= trace, 2=mild, 3=moderate, and 4= severe.

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