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Efficacy of topical mineralocorticoids in a rabbit model of ocular inflammation

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ABSTRACT

Objectives: The aim of this study was to determine the efficacy of mineralocorticoids in the treatment of ocular inflammation, whose potential use has not been assessed.

Methods: Thirty-five New Zealand albino rabbits were used in the study. Rabbits were divided into five groups. Only one eye was used for experimental purposes and the other eye was used as control. 11-deoxycortisol, deoxycorticosterone acetate, fludrocortisone acetate, aldosterone and 11-deoxycorticosterone were studied in a rabbit model of ocular inflammation. All animals in a group received the same corticosteroid. Paired t-tests and analysis of variance between subjects (ANOVA) were used to evaluate efficacy.

Results: The eyes treated with 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate had statistically significant lower fluorescence compared to control eyes. 11-deoxycortisol and deoxycorticosterone acetate provided a greater reduction in fluorescence compared to other corticosteroids.

Conclusions: Topical use of corticosteroids, especially those with mineralocorticoid activity can decrease ocular inflammation in a rabbit model. Clinical application of topical mineralocorticoids in human ocular inflammation needs to be performed.

Keywords: Topical mineralocorticoids, ocular inflammation, uveitis

Ocular inflammation is a common problem encountered in ophthalmology. Inflammatory responses in the eye are frequently seen in systemic diseases (e.g. rheumatoid arthritis), ocular diseases (e.g. infections), ocular surgeries (e.g. cataract extraction), or idiopathic conditions (e.g. anterior uveitis) [1-5].

The efficacy of glucocorticoids in treating ocular inflammation is well documented [6, 7]. In the treatment of ocular disease, this class of medications can be given topically. They can also be given through sub-tenon or intravitreal injections, with good ocular absorption [8-10]. Increased intraocular pressure is the primary complication of these medications [11].

The potential use of other classes of topical corticosteroids in the treatment of ocular inflammation has not been assessed, but they have theoretical utility. For example, mineralocorticoid receptors have been documented in rodentia ocular tissue [12] to maintain sodium-water homeostasis by regulating sodium channels [13, 14]. Through this role, the mineralocorticoids may impact eye function via modalities such as aqueous humor formation and corneal fibroblast formation [15, 16].

Given the anti-inflammatory properties of steroids and known intraocular receptors for mineralocorti-

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coids, it has been hypothesized that topical corticosteroids with primarily mineralocorticoid activity may also reduce intraocular inflammation. In this study, this hypothesis was tried to be tested.

This study had two specific aims: (1) to assess the reduction of ocular inflammation associated with topically administered mineralocorticoids in a rabbit model of prostaglandin-mediated uveitis, (2) to compare the efficacy of mineralocorticoids in reducing inflammation.

METHODS

Corticosteroid Solutions

We studied the following corticosteroids: 11-deoxycortisol, deoxycorticosterone acetate, fludrocortisone acetate, aldosterone, and 11-deoxycorticosterone. These compounds were obtained from Sigma Company (Sigma Chemical, St. Louis, MO).

Experimental Animals

Thirty-five New Zealand albino rabbits weighing between 2.5 and 3.5 kg were used in the study. Rabbits were treated in accordance with the principles of the Association for Research in Vision and Ophthalmology (ARVO) for the humane treatment of animals. Principles of laboratory animal care (NIH publication No. 85-23, revised 1985), the Office for Protection from Research Risks (OPRR) Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986), and the U.S. Animal Welfare Act were also followed. Only one eye was used for experimental purposes and the other eye was used as control. Slit-lamp and indirect fundoscopic examinations were performed on all eyes before and after euthanasia. No animals with pre-existing signs of ocular infection or inflammation were included in the study. The study was approved by Tulane University and conducted in the research laboratory of Tulane University Faculty of Medicine.

Rabbits were divided into five groups (Group 1:11-deoxycortisol; Group 2: Deoxycorticosterone acetate; Group 3: Fludrocortisone acetate; Group 4: Aldosterone; Group 5:11-deoxycorticosterone) with seven rabbits in each group (total of 35 rabbits, 70 eyes), based on corticosteroid use. All animals in a group received the same corticosteroid. All corticosteroids were administered topically as a 4 mg/ml solution.

Corticosteroid Administration

In this study, the experimental model described by Waterbury and Flach [17] was systematically followed. Accordingly, the rabbits were anesthetized with an intramuscular injection mixture of 1 ml of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (5 mg/kg). Corticosteroid solutions were applied to the right eye and saline was applied to the left eye. Both corticosteroid and saline solutions were administered four times per hour. The first drop application represented the time zero of the experiment. Drugs and saline were administered to the eyes as a single 50µL drop.

Dextran-isothiocyanate-fluoroscein FITC-dextran, (Sigma Chemical, St. Louis, MO) was used as a systemic marker in an anterior chamber fluorophotometric model to evaluate the impact of corticosteroids on inflammation [17]. A 20 mg/ml dose was prepared and injected into the marginal ear vein of each rabbit at hour 1.5 of the experiment. Endotoxin (2.5 µg/kg) isolated from Salmonella Typhimurium (Sigma Chemical) was injected into the same vein at hour 2, coinciding with the administration of drop three to initiate an inflammatory response.

The concentration of dextran-FITC in the anterior chamber was evaluated using a prototype flurophometer (OcuMetrics, Mountain View, CA) 90 minutes after endotoxin administration (30 minutes after the final drop application). This time frame is best to observe leakage of the fluorescent material [17]. The excitation wavelengths of this instrument are approximately 410 to 490 nm, with emission wavelengths of approximately 520 to 630 nm. The scanning optic head of the device is computer controlled. The computer also stores and processes all data.

Rabbits were put in a plexiglas holder and then placed in front of the instrument. Blue excitation light was focused on the eye of interest. The resulting fluorescence emitted by FITC-dextran was identified by a photomultiplier tube. Both right and left eyes were scanned.

Statistical Analysis

Paired t-tests were used to evaluate the effects of each corticosteroid versus saline control. Therefore,

each animal served as its control. Analysis of variance between subjects (ANOVA) was used to evaluate the efficacy of the corticosteroids compared to each other. Post-hoc analyses were done with Tukey's LSD. A p value <.05 was considered statistically significant.

RESULTS

Table 1 shows the mean levels of fluorescence emitted by Dextran-FITC in treatment and control eyes for the various corticosteroids. Eyes treated with 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate had statistically significantly lower fluorescence compared to control eyes ($p = 0.021$, $p = 0.003$, and $p = 0.042$; respectively). This suggested lower levels of leakage of dextran-FITC, and thereby, lower levels of inflammatory response in treatment eyes.

Table 2 shows the mean reduction levels in fluorescence seen for each of the corticosteroids. The reduction in fluorescence for each study animal was calculated by subtracting fluorescence in the treated eye from fluorescence in the control eye. Means for this difference were then calculated for each corticosteroid. Therefore, larger means indicate a greater reduction in fluorescence and suggest a greater reduction in the inflammatory response.

ANOVA results showed a significant difference in the mean fluorescence reduction among the mineralocorticoids [$F(6, 27)=3.95$, $p < 0.01$]. Tukey's LSD results revealed that 11-deoxycortisol and deoxycorticosterone acetate provided greater reductions ($p < 0.05$) in fluorescence compared to aldosterone and 11-deoxycorticosterone. Other pairwise comparisons were not statistically significant.

DISCUSSION

Of the five corticosteroids studied, none showed mineralocorticoid activity and only two showed glucocorticoid activity (minimal activity only, defined as $1/1000^{th}$ of the mineralocorticoid effect [18]). Our results showed that topically administered corticosteroids with little or no glucocorticoid activity reduced ocular inflammation in a rabbit model. Three of the topical corticosteroids produced a significant decrease in ocular inflammation in the experimental animals. 11-deoxycortisol, deoxycorticosterone acetate, and fludrocortisone acetate resulted in decreased endotoxin-induced fluorescein leakage into the anterior chamber compared to control eyes. Of these, 11-deoxycortisol and deoxycorticosterone acetate had only

Table 1. Mean anterior chamber fluorescence emitted by dextran-FITC in treatment versus control rabbit eyes

Mineralocorticoid	Right Eye (Treatment)		Left Eye (Control)		$t(4)$*	p value
	Mean (nm)	SD (nm)	Mean (nm)	SD (nm)		
Group 1 (11-deoxycortisol)	330.80	283.42	519.00	233.78	-3.67	**0.021**
Group 2 (Deoxycorticosterone acetate)	200.80	150.04	379.00	123.34	-6.35	**0.003**
Group 3 (Fludrocortisone acetate)	165.80	185.02	251.60	240.38	-2.94	**0.042**
Group 4 (Aldosterone)	247.80	180.56	113.61	113.61	0.25	0.815
Group 5 (11-deoxycorticosterone)	233.40	277.08	215.80	197.13	0.39	0.716

*t-test with 4 degrees of freedom

Table 2. Mean reduction in fluorescence by mineralocorticoid usage in rabbit eyes

Mineralocorticoid	Mean Reduction (nm)	SD (nm)
Group 1 (11-deoxycortisol)	188.20	114.25
Group 2 (Deoxycorticosterone acetate)	178.20	62.78
Group 3 (Fludrocortisone acetate)	85.80	65.35
Group 4 (Aldosterone)	-13.60	122.48
Group 5 (11-deoxycorticosterone)	-17.60	100.11

mineralocorticoid activity. The ocular anti-inflammatory properties of these mineralocorticoids are unique. Fludrocortisone acetate has both mineralocorticoid and glucocorticoid activity. However, in a model of inhibition of corneal angiogenesis, fludrocortisone was effective at dosages as low as 5 µg/ml, which was a dose where glucocorticoid activity would be minimal (Peyman GA, unpublished observation).

In comparing the different corticosteroids, neither aldosterone nor 11-deoxycorticosterone had a statistically significant effect on inflammation. Interestingly, aldosterone actually increased vascular inflammation when presented systemically [19, 20].

The rabbit model used in this study was used in other studies of ocular inflammation similarly [17, 21, 22]. The model was considered an appropriate estimate of human disease. Therefore, the results showed that topical use of a variety of corticosteroids, especially those with mineralocorticoid activity, could decrease ocular inflammation in a rabbit model.

The mineralocorticoid receptor (MR) may be active in the anterior segment of the eye, in particular in the iris and ciliary body epithelium of rat and human eyes. MRs are found in all cells that make up the blood-aqueous barrier and raises the question of their role in acute inflammatory conditions, such as uveitis [23].

There are publications in the literature showing that stimulation of mineralocorticoid receptors increases inflammation and plays a role in many vascular and inflammatory processes.

Long-term inappropriate or excessive MR activation was found to promote inflammation and increase oxidative stress markers in kidney and cardiovascular pathologies in humans as well as in animal models [24].

Bousquet *et al*. [26] demonstrated that aldosterone reduced the intensity of clinical inflammation in a dose-dependent manner in an experimental model of uveitis. The clinical benefit of aldosterone may be attributed to its being an MR antagonist [26].

Topical administration of Spironolactone-Loaded Nanomicelles, an MR antagonist, inhibited glucocorticoid-induced corneal wound healing in rabbits [27].

The mineralocorticoid receptor (MR) has a role in regulating retinal fluid homeostasis and also manages water and ion channel expression in Müller glial cells. In addition, the MR significantly contributes to the pathological mechanism behind central serous chorioretinopathy (CSCR) and it is thought to play a role in choroidal vascular bed relaxation.

Zhao *et al*. investigated the role of MRs in the development of CSCR, showing that intravitreal injection of glucocorticoids or aldosterone in rats propagated CSCR [25]. This was identified by choroidal vessel dilation and increased leakage and expression via the K channel KCa2.3 in ECs. This demonstrated that EC-MRs significantly contributed to the observed effects. Furthermore, subsets of patients thought to have chronic CSCR who were given oral eplerenone exhibited a marked improvement in visual acuity, which was linked to the resolution of choroidal vasodilation effects and even retinal detachments [25, 28].

Considering the results of these studies showing that MR stimulation increases inflammation, suppression of inflammation seems contradictory in our study. However, mineralocorticoids are likely to suppress inflammation using pathways and mechanisms similar to glucocorticoids. This may be dose-dependent or related to other unexplained mechanisms.

Limitations

The major limitation of the study is the lack of different corticosteroids. Further studies in large groups need to be performed to generalize the results, as our study was conducted with specific corticosteroids.

CONCLUSION

The ocular anti-inflammatory properties of corticosteroids with mineralocorticoid activity have not yet been determined. Our current results indicate the need for more comprehensive research on this class of corticosteroids for the treatment of ocular inflammation, e.g. reducing inflammation without increasing intraocular pressure. In addition, the clinical application of topical mineralocorticoids in human ocular inflammation needs to be established and it is an important topic to be investigated in future studies. In addition, more comprehensive studies are needed to explain the reasons for the results related to the suppression of inflammation, which is inconsistent with the literature.

Authors' Contribution

Study Conception: MK; Study Design: MK; Supervision: MK; Funding: Gholam A. PEYMAN; Materials: MK; Data Collection and/or Processing: MK; Statistical Analysis and/or Data Interpretation: MK; Literature Review: MK; Manuscript Preparation: MK and Critical Review: MK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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