

STEROID INDUCED OCULAR HYPERTENSION: AN ANIMAL MODEL

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ABSTRACT

Background: Steroids are common drugs that can lead to induction of glaucoma or ocular hypertension in humans. The aim of this study was to check whether steroids can be used to induce experimental glaucoma / ocular hypertension in an animal model.

Material & Methods: Betamethasone was used in an injectable dosage form. Injectable steroids took very short time to establish an animal model with raised intraocular pressure. Twenty-four healthy rabbits of a local strain weighing 1500 to 2000 grams were obtained and were kept at the animal house of the Department of Pharmacology Khyber Medical College Peshawar. The study was conducted on both eyes of 24 conscious rabbits. Two groups of animals were constituted i.e. Group A and B. Group A animals were made ocular hypertensive/ glaucomatous by injecting weekly sub-conjunctival betamethasone suspension. Group B served as the normal controls. It received no treatment during the entire research period.

Results: Steroid injection was found to raise the intraocular pressure very effectively and briskly. The difference between the intraocular pressures of the two groups was statistically significant.

Conclusion: It was concluded from this study that injectable suspension of betamethasone alone can induce ocular hypertension very effectively. It just took one week to raise the intraocular pressure. This animal model can confidently be used for research in pharmacology and ophthalmology to study the effects of newer drugs being tested for their anti-glaucoma effects.

KEY WORDS: Betamethasone; Glucocorticoids; Glaucoma; Ocular hypertension; Intra-ocular pressure.

This Article may be cited as: Khan SU, Ashraf M, Salam A, Ali Z, Rahman MU, Shah A. Steroid induced ocular hypertension: an animal model. *Gomal J Med Sci* 2014; 12:115-7.

INTRODUCTION

Experimental animal models have much improved our understanding of the etiology of different diseases including glaucoma. Investigators have been using rats, mouse and mice models which share some common ophthalmic properties.¹⁻³

Investigators have been successfully using rabbits as an animal model. The use of normotensive and ocular hypertensive rabbits is being done by the researchers for the last two decades. Conscious and unconscious rabbits can effectively be utilized for research purposes.⁴

Monkey, the primate model of glaucoma, is

considered the most relevant to human glaucoma.^{5,6} But it was beyond the scope of the present study to use either of above mentioned animal model. Firstly, due to unavailability of air puff tonometer in our setup and secondly, it was not practically at least possible to use monkey as an ideal glaucoma model. Therefore, rabbits were selected for induction of steroid induced ocular hypertension/ glaucoma for the present study.

There are so many techniques available for the induction of ocular hypertension in an animal model.^{6,7} It can also be done by using laser technology.⁵ Steroids induced raised intraocular pressure is also among the techniques in normal and conscious rabbits.^{4,8,9} Steroid induced raised intraocular pressure is reversible while laser induced injury leading to raised intraocular pressure is irreversible. Although, steroids can cause certain adverse effects if used for an extended duration of time.

Steroids have been known as an IOP elevating agents since 1950.¹⁰ Topical, oral or systemic admin-

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istration of any corticosteroid can produce a rise in intraocular pressure in otherwise normal eyes. This effect ordinarily requires several weeks of constant exposure and is more likely to result from topical than systemic administration.⁷

The aim of this study was to check whether steroids can be used to induce experimental glaucoma / ocular hypertension in an animal model.

MATERIAL AND METHODS

The study was conducted on both the eyes of 24 normal and conscious rabbits. Rabbits of either sex i.e. male and female and of both species i.e. albino and coloured strains were used. Age of rabbits was between 1-2 years and their weight was between 1.5-2.0 kg. They were kept under observation for 2 week before experimentation in the animal house of Khyber Medical College Peshawar. The animals were fed fodder, wheat grains and grams, ad libitum. Fresh and wholesome water was also provided ad libitum.

Two groups of animals were constituted. Each consisted of 12 rabbits. Group A: It was made ocular hypertensive. The rabbits of this group were injected Betamethasone suspension. Group B: This group was used as normal control i.e. normotensive. It received no treatment during the entire period of study. Proparacaine HCl 0.5% (Alcaine; Alcon – Couvreur, Belgium), Injection Betamethasone (Celestone Cronodose; Schering – Plough, Spain), Fluorescein Sodium 2% (Alcon – Couvreur, Belgium), Artificial tears drops (Alcon – Couvreur, Belgium), Perkins hand held appliation tonometer (Clement Clark Int. Ltd. Essex England), Rabbit boxes/ containers, Group A animals were made ocular hypertensive. The procedure to establish an animal model with raised intraocular pressure by using steroids in suspension form was as described by Melena et al in 1998. The rabbits were injected with a weekly sub-conjunctival suspension of betamethasone in both eyes. The volume of injection was 0.7 ml. The injection contained betamethasone sodium phosphate (3 mg/ml) and betamethasone acetate (3 mg/ml). This combination provides a readily available sodium phosphate and a slow released acetate fraction of betamethasone. All animals received sub-conjunctival injection of Betamethasone suspension in both eyes for 3 weeks. The injection schedule was 0,1,2 and 3.

The rabbits were held in specially designed wooden boxes. In the eye 1-2 drops 5% proparacaine HCl was instilled to induce local anesthesia. After a few minutes betamethasone suspension was injected in sub-conjunctival sac of the rabbits. Mild pressure was applied on the eye for a short period of time. To avoid diurnal variation of IOP, measurement was almost always started at the same time of the

day (9:00 AM) throughout the observational period. Measurement of the IOP in both eyes was performed, as a rule, twice a week. This helped to avoid corneal epithelial damage. The 1st measurement was taken immediately before injecting weekly Betamethasone i.e. Thursday and 2nd was recorded after 3 days i.e. Monday. Before starting the study, the IOP of all rabbits were taken for 2 weeks and 4 measurements of the IOP were taken during this time. Animals exhibiting fluctuations >5mm Hg in their IOP were excluded from the study (n = 5). New set of animals was included to replace the excluded ones. The values observed at “zero time” i.e. 1st injection of Betamethasone were considered the base line pressure. Before taking the measurements, the rabbits were given topical local anesthesia followed by fluorescein causing staining of cornea. Then, the animals were placed in specially designed containers. This caused the animals to remain unmoved. Then by applying appliation tonometer the IOP of the rabbits was recorded.

The intraocular pressure measurements of 24 rabbits were recorded. Statistical analysis was done by using SPSS version 12. All numerical values were represented as mean \pm SD. A p value of <0.05 was considered significant for all analysis.

RESULTS

The results of measurements of IOP of both the study group and controls are depicted in Table 1.

Table 1: Intra ocular pressures of ocular hypertensive (Group A) and normotensive rabbits (Group B).

T i m e Interval (Weeks)	Group B	Group A	p-value
0	21.00 \pm 0.20	20.83 \pm 0.75	>0.05
1	20.62 \pm 0.65	21.03 \pm 0.75	<0.05
2	20.70 \pm 0.30	21.75 \pm 0.30	<0.001
3	20.87 \pm 0.45	23.01 \pm 0.60	<0.001
4	20.92 \pm 0.58	26.51 \pm 0.22	<0.001
5	20.90 \pm 0.61	26.52 \pm 0.30	<0.001
6	21.00 \pm 0.50	26.51 \pm 0.24	<0.001
7	21.05 \pm 0.48	26.35 \pm 0.39	<0.001
8	21.07 \pm 0.37	25.35 \pm 0.31	<0.001

DISCUSSION

It has not yet been established that how steroids induce ocular hypertension or glaucoma. Several mechanisms have been proposed. It can cause destabilization of lysosomal membranes of

trabecular meshwork.. But, it is generally agreed that IOP elevation result from reduction in facility of aqueous humor outflow (Douglas H et al 2005, Basic and Clinical Science Course 2011-12).

The overall normal IOP (n=24) before the start of steroid treatment was in the range of 19.50 ± 0.75 to 21.75 ± 0.25 . Injectable steroid led to a rapid rise in IOP of the rabbits. The gain in IOP was found statistically significant after 2nd dose of betamethasone suspension ($p < 0.05$). The elevation became highly statistically significant after 4th injection ($p < 0.001$). Mean pre-steroidal baseline pressure was 20.83 ± 0.75 . The IOP in group A was in the range of 21.03 ± 0.75 and 26.52 ± 0.30 during research period.

The normotensive control, group B, did not show any statistically significant change in their IOP throughout the study period ($p < 0.05$). Their pressure was in the range of 20.62 ± 0.65 to 21.07 ± 0.37 .

After discontinuation of injection betamethasone some spontaneous IOP lowering was also noticed in group A. The drop in IOP was found statistically significant ($p < 0.05$) as compared to the values observed at the end of steroid therapy (Week # 3). After cessation of betamethasone therapy, the IOP was monitored for further 04 weeks in both groups. IOP lowering was observed during this period.

The general condition of the rabbit must be kept in mind as well. It is better to chose healthy and normal looking animals. During the entire research duration the normotensive and ocular hypertensive rabbits demonstrated tremendous survivability i.e. 100%. This excellent survivability can be attributed to fine weather conditions. The study was conducted during the months of March and April. It is expressed that extreme weather conditions i.e. cold or hot may affect animal's survivability. So, it is recommended that soft weather conditions should be chosen for research.

Use of topically applied prophylactic antimicrobials can be beneficial to protect the animals from opportunistic infections if they are being administered steroids for a longer duration. Pneumatic tonometer will be far better than applanation tonometer to measure the intraocular pressure. Thus only after fulfillment of these requirements can research time are saved and accurate research results be obtained.

CONCLUSION

It was concluded from this study that injectable suspension of betamethasone alone can induce

ocular hypertension very effectively. It just took one week to raise the intraocular pressure. This animal model can be used for research in pharmacology and ophthalmology to study the effects of newer drugs being tested for their anti-glaucoma effects.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE

None declared.