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Corticosteroid Induced Ocular Hypertension in Feline Model and Its Effects on White Blood Cell Count

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ABSTRACT: Prolong topical or systemic administration of corticosteroid produces undesirable side effects like increase intraocular pressure, lens opacities, neutrophilia, eosinopenia. This study was aimed at finding out the relationship between ocular hypertension and differential white blood cell count using corticosteroid induced ocular hypertension in feline model. A total of 5 cats were used for this study. The five cats were grouped into two (control and experimental). The control consisted of one cat while the experimental group consisted of four cats. The control group was left untreated while the experimental group was treated with topical 0.1% dexamethasone (steroid) 3-4 times daily. Blood samples were collected and analyzed before and after steroid instillation. At the end of the 4th week, the experimental group developed induced ocular hypertension with percentage increase of 83.5% from baseline IOP. Total WBC and neutrophil increased by 61.3% and 30.4% respectively, while lymphocyte and eosinophils decreased by76.2% and 20% respectively. This shows that there is a strong positive correlation between induced ocular hypertension, total blood cell and neutrophil count while there is negative correlation between induced ocular hypertension, lymphocyte and eosinophil count.

Keywords: Ocular hypertension, White blood cell count, Steroid, Feline model.

Introduction

Glaucoma is described as an ocular disease occurring in many forms having as its primary characteristics an unstable or a sustained increase in the intra-ocular pressure which the eye cannot withstand without damage to its structure or impairment of its function.
Open angle glaucoma is the second leading cause of blindness in Nigeria, African American and Hispanics in the US. Age, race, a positive family history, an elevated intra-ocular pressure (IOP) and corneal thickness have consistently been shown to be major risk factor in open angle glaucoma (Kragha, 1987).
Glaucoma has been linked to vascular origin and the influence of age upon inflammatory cell count and structure in glaucoma patient has been documented. Anemia and lymphocyte count has been suggested one time or the other as risk factors in glaucoma. Blood examination is performed for almost all patients with major illness because of the importance of determining the presence of anemia or leukocyte changes (Hall and Malia, 1986).
Increased intraocular pressure as a response to topically applied corticosteroids had been the subject of many clinical and experimental investigations. Furthermore several approaches had been utilized to study the effects of corticosteroids on ocular tissues. These comprise administration of steroids both to normal and inflamed eyes.
Various interpretations had been given to results due to differences in species, corticosteroids concentration dosages and route of administration. A clinically significant effect of corticosteroid utilization is an elevation of IOP. This response is more pronounced with topical than systemic administration of steroids in corticosteroid induced ocular hypertension.

It has been well established that anti-inflammatory glucocorticoids used for the treatment of ocular inflammatory diseases raise intraocular pressure (IOP) in normal and glaucomatous eyes this was reported in humans (Linner, 1959; Goldmann, 1912; Armaly, 1963; Becker, 1965;), in feline (Bhattacherjee *et al*., 1999; Zahn *et al*., 1992; Akinlabi, 2008; 2009), in bovine (Gerometta *et al*., 2004). It was also demonstrated in rabbits after sub-conjunctival injections (Hester, 1987), in children (Ng *et al*., 2000, Kwok *et al*., 1998), by systemic application on a child (Tham *et al*., 2004), and in chronic uveitis patients, (Ezra *et al*., 1997).

Comparative study of the intraocular pressure effects of fluorometholone versus dexamethasone shows that fluorometholone is less likely to increase intraocular pressure in corticosteroid responders than dexamethasone (Akingbehin, 1983).

Although corticosteroids is effective in the treatment of a wide range of inflammatory disorders, prolong topical or systemic administration of corticosteroid produces undesirable side effects like cushing's syndrome, increase intraocular pressure, lens opacities, neutrophilia, eosinopenia etc.During the last 35 years, the goal of anti-inflammatory therapy has been to achieve maximal anti-inflammatory effect with significantly reduced incidence of ocular side-effects.

Bigger *et al*. (1972) reported that peripheral blood lymphocytes from subjects with primary open angle glaucoma appeared more sensitive to glucocorticoids. Some investigators had reported the inhibitory effect of corticosteroids on leukocyte infiltration of aqueous humor (Bhattacherjee *et al*., 2003; Williams *et al*., 1987; Kulkani *et al*., 1981; Yamauchi *et al*. 1979; Howes *et a*l. 1994). The initial anti-inflammatory activity of the corticosteroids is related to an inhibitory effect upon the activity of polymorphonuclear leukocyte (PMNs) already within the tissues, which then prevents the ensuing cascade of characteristic inflammatory events (Williams *et al*., 1987).

Peng *et al*. (1999) assessed the effect of early dexamethasone therapy and blood cell count in preterm infants. Result revealed significantly higher numbers of segmented neutrophils and band forms and significantly lower numbers of lymphocytes and eosinophils. Akinlabi and Iyawe (2007) suggested further investigation of the eosinopenia, they found in the glaucoma group, when they compared hematological parameters between a group of open angle glaucoma patients and normals. Although the rapidity and extent of IOP elevation was found to exhibit considerable person to person differences, the fact that a very high percentage of glaucoma patients or suspects, exhibited more pronounced or more rapid steroid response than normotensive subjects prompted some investigators to consider topical steroid application as a provocative test for glaucoma.

Corticosteroids applications have been known to induce ocular hypertension which may be a predisposing factor to the development of glaucoma in certain susceptible individual. There had been suggestions that haematological parameters (differential white blood cell count) has a relationship with increased IOP. This study was therefore designed to investigate if there is any relationship between differential white blood cell count and increased IOP using steroid induced ocular hypertension in feline model.

Materials and Methods

This research is a prospective, randomized, controlled experimental study carried out in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. The cats were free of visible ocular lesions or defects when examined with the aid of ophthalmoscope and magnifying lens. The study was divided into two phases. First phase, the cats were allowed a minimum of 14 days to acclimatize to the new environment. At the end of adaptation period, their weight and baseline IOP were recorded. Also blood samples were collected and analyzed. Second phase was steroid instillation, collection of blood and analysis for any change in the differential blood cell count. The subject used for this study includes 5 adult cats of different breeds and of either sex bought from Benin City Edo State. The cats weight between 1.25 – 2.00 kg. The cats were acclimatized for some time so as to get them used to animal cage. The cats were fed mainly on fish and rice regularly 3 times daily. They have free access to water at all time. After the period of acclimatization, the cats were divided into two groups: Group I and Group II.

Group I were the experimental animals which consist of four cats. Group II was the control animal which consist of one cat. The animals were treated according to the University of Benin guidelines on animals used for experimental research.

Description of procedure: After the acclimatization period before steroid instillation, the cats were weighted and their average weight were determined. IOP were also measured for 5 days and their baseline pretreatment IOP were determined using hand held applanation tonometer. The pretreatment blood sample were collected and analysed to obtain the cats normal differential blood cell count. External examination of the cats eyes and also ophthalmoscopy were carried out to make sure that the cats were free from visible ocular lesions. During these time the cats were

made to feel free of any harm by touching and rubbing their body each time they were being fed. Then just before the treatment with corticosteroid, the cats were divided into 2 groups

Group I: The cats in this group were given 2 drops of topical 0.1% dexamethasone (0.1% maxidex) 4 times daily on both eyes

Group II: The cats in this group were left untreated on both eyes throughout the duration of the experiment. IOP measurement were carried out each week to determine the increase in intraocular pressure in response to corticosteroid instillation until there was established induced ocular hypertension. These was achieved on the 4th week of corticosteroid instillation. Then blood samples were again collected and analysed to determine any change in the differential white blood cell count.

Tonometry: To take IOP measurements each cat was made to lie on their sides with their eyes fixating upward. The cornea were anaesthesized using 0.4% Novesine then the eyes were stained with fluorescein strips. The tonometer was cleaned using methylated spirit. The IOP was taken when the eyelids were held apart with the free hand while holding the tonometer with the other hand. The IOP reading were taken when the mires were aligned which were taken twice to get accurate reading. The cats eyes were irrigated to check for any abrasion of the cornea then lastly application of 0.5% chloramphenicol eye drop to prevent infection.

Ophthalmoscopy: Ophthalmoscopy was carried out before the treatment to rule out any structural and ocular lesions and after the treatment to determine any change in the structure of the cats eyes by the induced ocular hypertension.

Collection of Blood Sample: Blood samples of the cats were collected for the differential blood cell counts before and after corticosteroid treatment.2 ml of venous blood were collected through the ear lobe of the cats. Prior to the collection of blood, the ear lobe were sterilized by cleaning with cotton wool soaked in methylated spirit which also help in making the vein prominent. The blood samples collected were put in EDTA bottles. The blood sample collected were taken to University of Benin Teaching Hospital chemical pathology laboratory immediately. Here the blood samples were placed on slides and smeared with the use of another slide, after this the slide was allowed to dry and then they were stained. At this stage the prepared slide were ready for reading and it was taken to the binocular microscope for counting of the number of cell.

Results

A total of 5 cats were used in this experimental study. The weight of the cats range between 1.25 – 2.00 kg with a mean weight of 1.63 kg. The data obtained in this study were analyzed by calculating the mean, variance, standard deviation and percentages of the data collected. ANOVA and t-test (LSD) were used to test for significant difference between baseline and corticosteroid instillation in IOP and differential white blood cell count. Pearson correlation coefficients was used to determine the relationship between change in differential while blood cell count and induced ocular hypertension.

Table I: Mean IOP reading for 5 cats

Cat	Mean IOP before steroid (mmHg)	Mean IOP before steroid (mmHg)	Change in IOP from mean baseline (mmHg)	% change in IOP mean baseline
Group I				
1	14.60	22.30	7.7	52.7
3	15.10	25.30	10.2	67.5
4	14.30	25.80	11.5	80.1
5	15.10	27.80	12.7	84.1
Mean	14.80	25.3	10.5	70.9
Group I				
2	15.50	15.00	0.50	3.2

Table 2a: White blood cell before steroid instillation

Cat	Total WBC/ul	Total WBC/ul	Lymphocyte%	Eosinophil%	Monocyte%	Basophil%
Group I						
cat 1	6,500	70	28	2	-	-
cat 3	9,400	58	41	1	-	-
cat 4	10,.900	75	24	1	-	-
cat 5	10,700	83	16	1	-	-
Group II						
cat 2	25,300	87	13	-	-	-

Table 2b: White blood cell after steriod instillation

Cat	Total WBC/ul	Total WBC/ul	Lymphocyte %	Eosinophil%	Monocyte%	Batophil %
Group 1						
cat 1	26,000	93	6	1	-	-
cat 3	14,600	96	4	-	-	-
cat 4	10,100	93	7	-	-	-
cat 5	9,800	91	9	-	-	0
Group II						
cat 2	25,000	80	15	-	-	-

Table 3: Mean distribution and percentage change of WBC before and after steroid instillation

	Mean WBC before steroid instillation	Mean WBC after steroid instillation	% change in WBC analysis	Level of significance
Total WBC/ul	9,375.00	15,125.00	61.33	0.1929
Neutrophil %	71.500	93.250	30.42	0.0065
Lymphocytes %	27.250	6.500	76.15	0.008
Eosinophil %	1.250	1.000	20.00	0.685
Monocyte %	-	-	-	-
Basophil %	-	-	-	-

Table 4: Comparison between induced IOP and changes in WBC parameters

WBC Parameter	Mean	Variance	Standard deviation
IOP (mmHg)	20.038	30.847	5.554
Total WBC/ul	12250	35808256.00	5984.000
Neutrol %	82.375	183.982	13.564
Lymphocyte %	16.875	171.558	13.098
Eosinophil %	1.2	0.200	0.447
Monocyte %	-	-	-
Basophil %	-	-	-

Table 5: Relationship between induced ocular hypertension and change in differential white blood cell count analysis

Combination of IOP and WBC parameter	Correlation value	Probability
IOP and total WBC	0.513	0.1936
IOP and neutrophil	0.882	0.0039
IOP and lymphocyte	-0.873	0.0047
IOP and Eosinophil	-0.205	0.7407

Data analysis: Topical ocular administration of dexamethasone, induced ocular hypertension in the experimental cats (group 1). The mean baseline IOP value of 14.80 (± 0.966) mmHg was increased to a mean of 25.275 (± 2.298) mmHg with, a percentage increase of 70.8% (Table 1). The result showed a statistically significant difference between baseline IOP and IOP of steroid treated cats. ($p < 0.001$). There was no significant difference in the IOP untreated cat (group 2) analysis revealed that corticosteroid induces ocular hypertension.

The mean and percentage change of leukocytes and differential white blood cell count before and after steroid instillation is shown in tables 2 and 3. Total WBC and neutrophil increases to 61.3% and 30.4% respectively, while lymphocyte and eosinophil decreases to 76.2% and 20% respectively. ANOVA and t - test also revealed a statistically significant difference in differential white blood cell count analysis before and after steroid instillation (for total WBC and eosinophil ($p > 0.05$),while for neutrophil and lymphocyte ($p < 0.05$)).

Tables 4 and 5 show the relationship between induced ocular hypertension and change in differential white blood cell counts. Pearson correlation coefficients statistical analysis revealed that total WBC and neutrophil has a positive relationship with induced ocular hypertension. This means that increase in IOP causes increase in Total WBC and neutrophil count. While lymphocyte and eosinophil has a negative relationship with induced ocular hypertension. This means that increase in IOP causes decrease in lymphocyte and eosinophil count.

The first hypothesis that there is no significant difference between the differential white blood cell count analysis of corticosteroid induced ocular hypertension and baseline intraocular pressure in the feline model was rejected ($p < 0.05$) as there was a change in WBC parameters.

The second hypothesis that there is no significant relationship between the differential white blood cell count analysis and induced ocular hypertension (glaucoma) was rejected ($p < 0.05$).

Discussion

The increase in IOP of cats treated with 4 times daily topical dexamethasone shows a statistically significant difference ($p<0.001$) from the baseline after 4 weeks of treatment. This corroborates the works of earlier investigators (Bhattacherjee *et a*l., 1999; Zahn *et al*., 1992; Akinlabi *et al*., 2008, 2009). It is known that steroid-induced IOP elevation is secondary to increased resistance to aqueous outflow. Some evidence indicates that the defect could be increased accumulation of glycosaminoglycans or increased production of trabecular meshwork-inducible glucocorticoid response (TIGR) protein, which could mechanically obstruct outflow (Lutgen-Drecoll *et al*., 1998). Other evidence points toward corticosteroid-induced cytoskeletal changes that could inhibit pinocytosis of aqueous humor or inhibit the clearing of glycosaminoglycans, resulting in the accumulation of this substance (Tian and Kaufman, 2012). The change in differential white blood cell count in corticosteroid induced ocular hypertension also revealed statistically significance difference in neutrophil and lymphocyte count ($p < 0.05$) while total white blood cell count and eosinophil count were not statistically significant ($p > 0.05$) even though there was a change in mean values.

The correlation analysis revealed that total white blood cell and neutrophil count has a strong positive relationship with induced ocular hypertension with a correlation value of 0.5129 and 0.8801 respectively. Total WBC was not significant ($p > 0.05$) while neutrophil is significant ($p<0.05$). The correlation analysis also revealed that lymphocyte and eosinophil has negative relationship with induced ocular hypertension with a correlation value of– 0.8729 and – 0.205 respectively lymphocyte was significant ($p < 0.05$) while eosinophil was not significant ($p > 0.05$).

Positive relationship means that when intraocular pressure increases, total WBC and neutrophil count increases as well. While negative relationship means that when intraocular pressure increases, lymphocyte and eosinophil count decreases and vice versa. This finding showed that ocular hypertension has a relationship with differential WBC.

This relationship between IOP and WBC count was in line with that of Bhattacherjee et al. 2003 which reported that in feline eyes, Al-2512 significantly ($p < 0.05$) inhibited lymphocyte infiltration of aqueous humour by 59%, compared to 37% inhibition by prednisolone acetate. This is consistent with our findings which showed decreased lymphocyte count in cats treated with topical 0.5% dexamethasone.

The fall in lymphocyte number that usually occurs 61 to 120 hours after birth seemed to be accentuated in the treatment group. However, the lymphocyte count remained comparable between the group throughout most of the trial until day 21 to day 28 when the treated group had a significantly lower lymphocyte count. Kavelaars *et al*.,(1995) noted that the immune system of the newborn is more sensitive to dexamethasone inhibition of T-cell proliferation than the adult immune system, and proposed that this increased sensitivity to glucocorticosteroids may compensate the low levels of circulating glucocortico steroids.

The eosinophil count tended to be higher in the control group throughout the trial, though the difference is not significant. This finding corroborates earlier report of eosinopenia in glaucoma patients (Akinlabi and Iyawe, 2007).

Prostaglandin (PG) endoperoxidase synthase, known as cyclooxygenase(COX) is the rate-limiting enzyme in the biosynthesis of PG. There are two distinct enzymes, COX-1 and COX-2. While COX-1 is constitutively expressed in most cells, the expression of COX-2 is generally low under basal conditions but is usually induced by inflammatory mediators, mitogens and growth factors(Pujols *et al* (2009).

Corticosteroids prevent the up-regulation of the cyclooxygenase II enzyme which may be more relevant to chronic inflammatory states (Fernandez- Morata et al 2000). Corticosteroids are more effective inhibitors of cell influx because of their ability to inhibit up-regulation of pro-inflammatory cytokines, chemokines, and adhesion molecules involved directly in leukocyte activation, rolling, and diapedesis(Williams *et a*l 1987).

Pujols *et al* (2009) found a weak but significant negative correlation between COX-2 mRNA levels and eosinophil counts,in nasal polyps in vivo after glucocorticoid treatment. They concluded that, changes in COX-2 mRNA induced by glucocorticoid treatment may in part, be a consequence of changes in the tissue cell composition.

Conclusion

The result from the analysis of the data obtained from this study revealed that corticosteroid – induced ocular hypertension has effect on differential white blood cell count. This result also shows that there is a strong positive correlation between induced ocular hypertension, total blood cell and neutrophil count while there is negative correlation between induced ocular hypertension, lymphocyte and eosinophil count. Our suggestion is that further investigation needs to be done on the eosinopenia observed in corticosteroid induced ocular hypertension.

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