Glaucoma Experimental Induction in Rabbits New Zealand

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Abstract: Three methods of induction were compared of glaucoma experimentally in rabbits based on the increase of the pressure intraocular (PEEP) and macrocospic changes (edema, atrophy). Males of race used 15 rabbits New Zealand with an average weight of 2.730 kg, divided in three treatments with five repetitions, to which they administered the following solutions; T1: viscoelastic (hyaluronate of sodium), T2: phenol in oil of almond and T3: erythrocytes, in the previous chamber of the left eye, using the right eye as witness. The period of follow-up was carried out at 9:00 and 19:00 hours for ten days. With regard to the PIO there were no differences between the procedures of induction, not in the schedules of measurement ($P < 0.05$). The treatment based on phenol in oil of almond, produced major problems and disabled significantly the percentage of rabbits that presented reflection pupilar ($P < 0.05$). The blindness associated with the problems of edema and atrophy ($P < 0.01$), but not to the pressure intraocular. None of the evaluated procedures I manage to induce the glaucoma.
[Romero Núñez C, Reyes Clímaco L, Campos García Rojas C, Pineda Cruz J, Mendoza Barrera GE, Mendoza Barrera EA, Bautista Gómez LG, Hernández García PA, Heredia Cardenas R. **Glaucoma Experimental Induction in Rabbits New Zealand.** *Life Sci J* 2017;14(3):97-100]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 15. doi:10.7537/marslsj140317.15.

Key words: rabbits; glaucoma; intraocular pressure; hyaluronate of sodium; erythrocytes.

1. Introduction

The glaucoma is defined as an optical, bilateral, chronic neuropathy and multifactorial, characterized by atrophy of the nerve optical and loss of the field of vision, which represents a common end of different conditions that can concern the eye, as the increase of the pressure intraocular (Morrison *et al*., 2008). At present it is considered that they exist two principal forms of glaucoma: of opened angle and of closed angle. The primary glaucoma of opened angle is characterized by the high pressure intraocular, which favors the development and advance of the disease. Generally there are no symptoms until the optical nerve is damaged and the peripheral vision gets lost (Zanón and Pinazo, 2008). The primary glaucoma of closed angle is a pathology in which an increase produces to itself of PIO rapid as proved from the obstruction of the drainage of the watery humor for the partial or complete closing of angle of the previous chamber for the iris (Bautista and González, 2008). This disease has been considered to be the first reason of irreversible blindness and constitutes the second reason of blindness worldwide in persons (Landín *et al*., 2009), Likewise the glaucoma represents 33% of the principal motives of consultation in the ophthalmological veterinary clinic (Fuchs *et al*., 2004).

Several models have been brought for the induction of glaucoma, using different solutions. In rabbits it has been achieved to induce glaucoma by means of the inoculation subconjuntival of phenol to 5% in oil almond, managing to increase the pressure intraocular (Ghada *et al*., 2010). Also they have been used erythrocytes to induce glaucoma, simulating what happens in a hemorrhage in the previous chamber of the eye where the hemolysed erythrocytes obstruct the flow of the aqueous humor (Ocando *et al*., 2009).

In the investigation of the glaucoma, the studies of animal model play an important paper to understand the mechanisms of formation and evacuation of the watery humor, as well as the maintenance of the homeostasis of the pressure intraocular; these tests have allowed to know better the etiology of the glaucoma and the development of new treatments (Lim *et al*., 2005; Vecino, 2008). The albino rabbit New Zealand has been used widely in this type of studies by his easy managing in the laboratory (Medrano *et al*., 2010). Considering the importance that has the experimental development of this disease, the aim of this experiment was three methods of induction compared of glaucoma in rabbits New Zealand, by means of different substances; hyaluronate of sodium, phenol in oil of almond and erythrocytes.

2. Material and methods

For the induction of glaucoma, males of race used 15 rabbits New Zealand with an average weight of 2.730 kg, which were randomized in three treatments based on the administration of different solutions:

T1: 0.1 ml of a viscoelastic to hyaluronate of sodium to 1.6% Biovisc, based on Garzon and Morales, (2003).

T2: 0.3 ml of a solution of phenol to 5 % in oil of almonds, as Ghada *et al*., (2010).

T3: 0.2 ml of erythrocytes to 2%, the preparation of the solution consisted of the compilation of 2 ml of blood of the ear vein of every rabbit, which was centrifuged to 800 *g* during 10 minutes, the supernatant discarded and the remaining solution was diluted by saline physiological solution (0.2ml) (Ocando *et al*., 2009).

Before the application of the above mentioned treatments, one administered as anesthesic 3 mg/kg/IM of xylazine, 35 mg/kg/IM of ketamine and 2 drops of tetracaine hackneyed, to a concentration of 5 mg/ml.

The administration of treatments was carried out by means of the centesis in bevel of the previous chamber of the left eye by a needle of 0.50 by 16 mm. Whereas the right eye was handled as witness, to alone whom I him inoculate 0.1ml of physiological solution to 0.9%.

The experiment had a duration of 10 days, where the measurement of the pressure registered intraocular two times for day (9:00 and 19:00 hours), which carried out with Schiotz's tonómetro. In the same way a valuation was effected ophthalmoscopic by means of an Ophthalmoscope (WA11730) for the incursion of the presence of macrocospic changes (corneous edema, atrophy of the iris, mydriasis hinge, blindness) as well as the photomotive reflection pupilary. All the procedures were supported by the Committee of Ethics of Agricultural Sciences of the Autonomous Metropolitan University.

The results of pressure intraocular were analyzed in agreement to a design completely at random by arrangement factorial (4 x 2) with measurements repeated (ten days) where the factors were based on the treatments of induction and the periods of measurement. The percentages between the treatments and the macrocospic changes compared with the test Kruskal Wallis (Herrera and García, 2011). Also there was realized an analysis of logistic regression, taking the percentage of blindness as a dependent variable in order to evaluate the effect of the pressure intraocular and other microscopic changes (Marasini *et al*., 2012).

3. Results

With regard to the induction of the pressure intraocular, there was no effect of the administered treatments and the periods of measurement (Table 1). None of the treatments managed to increase the pressure intraocular ($P > 0.05$) neither as well as were differences in the schedules of measurement registered ($P > 0.05$).

The treatment with phenol in oil of almond, caused major problems in the treated eyes and disabled significantly the reflection pupilar ($P < 0.05$) (Table 2). The inoculation of red blood cells in the previous chamber showed major frequency of blindness and of atrophy but it was not significantly different from the group witness (Table 2). The results of the logistic regression indicate that the blindness associated to the problems of edema and atrophy ($P < 0.01$), but not to the pressure intraocular (Table 3).

4. Discusion

On the basis of that the glaucoma is a degenerative disease caused by the increase of the pressure intraocular (PEEP), none of the treatments was effective to reproduce the problem. Rainer *et al*., (2006) they evaluated two viscoelastics to induce the PIO after the incision of the surgery of cataract; methylcellulose hydroxypropyl to 2% and Viscostat, hyaluronate of sodium to 3% with condroitin sulfate to 4% (similar to the T1 of this study) at postoperatory 6 a.m. the PIO was high in the group of persons to which Viscostat managed the affairs, but after a postoperatory week there were no differences in PEEP. With regard to the results of this test, it was hoping that the Biovisc was increasing the PIO since it has a major molecular weight (2'500.000 Daltons) compared to used in Rainer's study *et al*., (2006).

The administration of phenol in oil almond, the PIO was kept higher in three weeks post treatment (Ghada *et al.,* 2010), in the same way they inoculated the solution in four quadrants of the eye, which could make the response more effective in PEEP.

It is clear that the treatment with phenol though it did not manage to increase the PIO, was the one that caused major hurts in the treated eyes. It is known that the phenol in solutions diluted (from 1 to 2%) can cause severe burns, if the contact is prolonged, whereas the constant exhibition can cause conjunctivitis and opaqueness of the cornea. It is known that the hyphema is associated with the glaucoma (Migueli and Ortiz, 2003), which justified the employment of the erythrocytes solution.

In an experiment realized for Ocando *et al*., (2009) they managed to induce glaucoma in rabbits by means of the erythrocytes solution, nevertheless it did not increase the PIO. It has been achieved to cause glaucoma secondarily in rabbits on having injected into the previous chamber human erythrocytes of cells sickled due to the curled form, the red blood cells sickled cannot happen across the capillaries and the

venules, therefore some associate with others and cause obstructions increasing the PIO (Goldberg, 1979).

5. Conclusión

None of the evaluated treatments could reproduce glaucoma, the method with phenol in oil of almond was the one that caused major hurts, it would be suitable to study variants as distribution of the solution and concentration of phenol to manage to reproduce the glaucoma in consistent form.

Table 1. Effect of the treatments of induction of pressure intraocular and the periods of measurement.

Factor	Pressure intraocular (mmHg)
Treatment	
Witness	20.51
Viscoelastic	20.33
Phenol almond	20.97
Erithrocytes	19.93
Hour of record	
9:00 h	19.79
19:00 h	21.13
SEM	0.419

There were no differences between the treatments and the hour of record ($P > 0.05$).

Table 2. Frequency of injuries (%) in different treatments for induction of glaucoma.

Treatment	Corneous edema	Atrophy of the iris	Mydriasis fixes	Blindness	Photophobia	Photomotive reflection pupilar
Witness	0.0b	0.0b	0.0b	0.0b	0.0a	100.0a
Viscoelastic	0.0b	0.0b	0.0b	0.0b	4.0a	100.0a
Phenol almend	94.0a	80.0a	58.0a	90.0a	10.0a	48.0b
Erithrocytes	0.0b	4.0b	0.0b	14.0b	0.0a	100.0a
It tries Kuskal Wallis's						
Square Ji	17.3	15.79	9.38	14.95	1.91	9.38
Degrees of freedom	3	3	3	3	3	3
Probability	0.0005	0.0012	0.0246	0.0019	0.5911	0.0246

a,bLiteral different inside column they are different ($P < 0.05$).

Table 3. Analysis of logistic regression on the frequency blindness (%) in rabbits.

Parameter	Degrees of freedom	Esteeming	Standard mistake	Ji-squared of Wald	P Ji squared
Intercept	1	0.7042	2.4004	0.0861	0.7693
Pressure intraocular mm Hg	1	0.0835	0.0824	1.0273	0.3108
Edema %	1	-5.2361	1.2810	16.7068	0.0001
Atrophy %	1	-2.6045	1.0258	6.4459	0.0111

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3/25/2017