

Artículo de Investigación

**Variation of intraocular pressure comparing rebound (TONOVET Plus®) and applanation (TONO-PEN VET®) tonometry in New Zealand rabbits treated with Amlodipine®**

*Variación de la presión intraocular comparando la tonometría de rebote (TONOVET Plus®) y applanación (TONO-PEN VET®) en conejos de Nueva Zelanda tratados con Amlodipino®*

*Variação da pressão intraocular comparando a tonometria de rebote (TONOVET Plus®) e de applanção (TONO-PEN VET®) em coelhos da Nova Zelândia tratados com Amlodipina®*

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**Abstract**

Rabbits have played an important role in ophthalmological research processes, however in the environment there has been a deficiency of sensitive equipment in measurement of intraocular pressure for this species. To compare intraocular pressure (IOP) measured with rebound (TONOVET Plus®) and applanation (TONO-PEN VET®) tonometry in healthy New Zealand rabbits treated with Amlodipine. Twelve healthy male New Zealand white rabbits, 5 months old; two groups with 6 individuals were used in this study. The experimental group was given Amlodipine® at a dose of 5 mg/kg, once a day for a month. Blood pressure and intraocular pressure were measurement before treatment and 6 h after treatment in a period of one month. A trend is noted

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where the group of animals treated with amlodipine® presents lower values than the control group, both in systolic and diastolic pressure. The systolic pressure of the control group showed an average of  $116.5 \pm 7.9$  mmHg and  $117.4 \pm 8.9$  mmHg and the treatment group showed an average of  $113.6 \pm 7.9$  mmHg and  $111.8 \pm 10.0$  mmHg (pretreatment and posttreatment respectively). Tonometry: the values obtained using the TONOVET Plus® ( $19 \pm 3$  mmHg) are on average higher than the values obtained using the TONO-PEN VET®

( $17 \pm 3$  mmHg). It was possible to demonstrate the relationship between systemic blood pressure and intraocular pressure in New Zealand white rabbits. Rebound tonometry showed less variability in results than applanation tonometry. This study confirmed that both tonometers are reliable for measuring IOP in rabbits.

**Keywords:** *glaucoma; hypertension; intraocular pressure; iridocorneal angle; tonometer; tonometry.*

## Resumen

Los conejos han jugado un papel importante en los procesos de investigación oftalmológica, sin embargo, en el medio ha existido una deficiencia de equipos sensibles en la medición de la presión intraocular para esta especie. Para comparar la presión intraocular (PIO) medida con la tonometría de rebote (TONOVET Plus®) y applanación (TONO-PEN VET®) en conejos Nueva Zelanda sanos y tratados con Amlodipino, fueron usados doce conejos Nueva Zelanda, machos, sanos, de 5 meses de edad y distribuidos en dos grupos con 6 individuos cada uno. El grupo experimental recibió Amlodipino® en una dosis de 5 mg/kg, una vez al día durante un mes. La presión arterial y la presión intraocular se midieron antes del tratamiento y 6 h después del tratamiento en un período de un mes. Se observa una tendencia donde el grupo de animales tratados con amlodipino® presenta valores más bajos que el grupo control, tanto en presión sistólica como diastólica. La presión sistólica del grupo control mostró un promedio de  $116,5 \pm 7,9$  mmHg y  $117,4 \pm 8,9$  mmHg y el grupo de tratamiento mostró un promedio de  $113,6 \pm 7,9$  mmHg y  $111,8 \pm 10,0$  mmHg (pretratamiento y postratamiento respectivamente). Tonometría: los valores obtenidos con el TONOVET Plus® ( $19 \pm 3$  mmHg) son en promedio superiores a los valores obtenidos con el TONO-PEN VET® ( $17 \pm 3$  mmHg). Fue posible demostrar la relación entre la presión arterial sistémica y la presión intraocular en conejos blancos Nueva Zelanda. La tonometría de rebote mostró menos variabilidad en los resultados

que la tonometría de aplanamiento. Este estudio confirmó que ambos tonómetros son confiables para medir la PIO en conejos.

**Palabras clave:** *ángulo iridocorneal; glaucoma; hipertensión; presión intraocular; tonómetro; tonometría.*

## Resumo

Os coelhos têm desempenhado um papel importante nos processos de pesquisa oftalmológica, porém no meio tem havido uma deficiência de equipamentos sensíveis na medida da pressão intraocular para esta espécie. Comparar a pressão intraocular (PIO) medida com tonometria de rebote (TONOVET Plus®) e de aplanção (TONO-PEN VET®) em coelhos saudáveis da Nova Zelândia tratados com Amlodipina®. Foram utilizados neste estudo doze coelhos brancos da Nova Zelândia, machos saudáveis, 5 meses de idade; distribuído em dois grupos com 6 indivíduos cada um. O grupo experimental recebeu Amlodipina® na dose de 5 mg / kg, uma vez ao dia durante um mês. A pressão arterial e a pressão intraocular foram medidas antes do tratamento e 6 horas após o tratamento em um período de um mês. Nota-se uma tendência onde o grupo de animais tratados com amlodipina® apresenta valores inferiores ao grupo controle, tanto na pressão sistólica quanto na diastólica. A pressão sistólica do grupo controle apresentou média de  $116,5 \pm 7,9$  mmHg e  $117,4 \pm 8,9$  mmHg e o grupo tratamento apresentou média de  $113,6 \pm 7,9$  mmHg e  $111,8 \pm 10,0$  mmHg (pré-tratamento e pós-tratamento, respectivamente). Tonometria: os valores obtidos com o TONOVET Plus® ( $19 \pm 3$  mmHg) são em média superiores aos valores obtidos com o TONO-PEN VET® ( $17 \pm 3$  mmHg). Foi possível demonstrar a relação entre a pressão arterial sistêmica e a pressão intraocular em coelhos brancos da Nova Zelândia. A tonometria de rebote mostrou menos variabilidade nos resultados do que a tonometria de aplanção. Este estudo confirmou que ambos os tonômetros são confiáveis para medir a PIO em coelhos.

**Palavras-chave:** *ângulo iridocorneal; glaucoma; hipertensão; pressão intraocular; tonómetro; tonometria.*

## Introduction

Some optic neuropathies cursing with the degeneration of ganglion cells and their respective axons in the retina lead to changes in the optic disc and in some cases; even loss of vision knows glaucoma. After diabetic retinopathy, glaucoma is the second cause of blindness in humans and the third in canines <sup>(10,19,31)</sup>. Glaucoma is classified depending on the characteristics of the iridocorneal angle as an open angle or closed angle or, depending on the origin, as primary or

secondary. One of the most common types of this disease in humans is primary open-angle glaucoma, however in canines we find angle-closure glaucoma more frequently <sup>(10,31)</sup>. Intraocular pressure (IOP) represents the primary risk factor for developing glaucoma <sup>(6,13,19)</sup>. IOP depends on the flow rate of aqueous humor and its resistance to outflow; thus, the result of the relationship between intraocular fluid, elasticity and stiffness of the cornea and sclera would be called intraocular pressure <sup>(6,10,29)</sup>.

Aqueous humor is a hydatoid fluid, which is formed by a passive ultrafiltration process and by an active secretion of the epithelium that covers the ciliary body. The constant flow of aqueous humor supplies nutrients to the avascular cornea and lens, also removes metabolic waste. The level of production of the aqueous humor should be equal to its level of output. The formation of aqueous humor depends on ciliary arterial blood pressure, which is equal to the intraocular pressure <sup>(29)</sup>. Aqueous humor circulation begins when the fluid produced in the posterior chamber passes through the pupil and enters the ciliary opening, which contains the trabecular meshwork. The mesh filters this liquid to reach the vessels of the scleral venous plexus and then from there to the venous system <sup>(6,10,25)</sup>.

The procedure to measure intraocular pressure is called tonometry, which can be indentation, applanation or rebound <sup>(20,24,28)</sup>. With tonometry, more precise IOP data can be obtained, with indentation tonometry being the most imprecise of all. Elements such as the thickness and biomechanics of the cornea; as well as edematous, ulcerative and pigmentary alterations can affect the determination of IOP by introducing noise in the tonometry <sup>(3,4,7)</sup>.

Systemic blood pressure has been documented to influence intraocular pressure, becoming a risk factor for the development and progression of glaucoma. On the one hand, the microvasculature of the retina, the choroid and the optic nerve can present a series of pathophysiological changes that can manifest in conditions such as retinopathy, choroidopathy and hypertensive optic neuropathy <sup>(9,10,31)</sup>. Alternatively, it has been shown that the nocturnal arterial hypotension that some patients present with favors glaucomatous optic neuropathy progressively due to a reduction in the perfusion of the optic nerve head, leading to a greater deterioration of the visual field <sup>(6,13,17)</sup>.

Calcium antagonist drugs are effective for treating cardiovascular diseases, including high blood pressure. Calcium enters excitable cells through voltage-gated calcium channels <sup>(8)</sup>. Amlodipine is a third-generation drug, dihydropyridine calcium antagonist, selective for vascular smooth muscle, with little effect on cardiac contractility; it exerts its action through the inhibition of

calcium entry into vascular smooth muscle and myocardial cells, decreasing peripheral vascular resistance. It has an elimination half-life of 40 h (h) to 60 h, which confers several pharmacokinetic characteristics that are not seen with other calcium antagonist drugs. The bioavailability of the orally administered drug is between 60% and 80% and accumulates to a steady state with administration once daily for a period of approximately 1 to 1 and a half week <sup>(8,26)</sup>.

Due to the devastating impact that glaucoma has on the quality of life of animals, more efforts are required to understand this pathology and develop new therapeutic and prevention options. Rabbits have played an important role as an animal model in ophthalmological research processes, allowing the development of surgical techniques, as well as in the evaluation and monitoring of new therapeutic options <sup>(8,16,33)</sup>. However, in the environment there has been a deficiency of sensitive equipment in the measurement of intraocular pressure for this species. Therefore, we set ourselves the objective of comparing rebound and applanation tonometry in healthy New Zealand rabbits treated with Amlodipine.

## Materials and methods

### Animals

Following the arrive guidelines, twelve healthy male New Zealand white rabbits, 5 months old and an average weight of 3.1 kg were used in this experimental study. Which were raised under convectional bioterium conditions and handled according to the institutional guidelines of the Central Animal Facility, individually housed in a cage with controlled temperature and humidity conditions, rabbit's commercial food and water ad libitum.

Two groups treated (T) and control (C) were randomized, each group with six individuals (12 eyes per group for each measurement). The treated group received amlodipine at a dose of 5 mg/kg <sup>(15)</sup> diluted in 2-mL water administered orally, once a day for a month. The control group received only 2-mL water,

### Blood pressure

A Contec<sup>®</sup> veterinary blood pressure monitor with a neonatal cuff was used to blood measure in the left forelimb (brachial/radial artery) before treatment and 6 h after treatment on days 1 – 4 – 8 – 12 - 16 - 20 - 24 - 28 and 32. Systolic and diastolic blood pressure was recorded in both groups.

## Tonometry

For measuring intraocular pressure, the rabbits were gently manipulated without applying pressure to the neck or eyelids. A rebound tonometer (TONOVET Plus<sup>®</sup>, Nasdaq, Helsinki) and an applanation tonometer (TONO-PEN VET<sup>®</sup>, Buffalo, New York) were used following the indications of each manufacturer. It was started using a rebound tonometer and then the applanation one for IOP measurement (after 1 min of topical application of a drop of proparacaine hydrochloride ophthalmic solution, Alcaïne<sup>®</sup>), maintaining a methodical order starting with the left eye and then the right. The intraocular pressure and blood pressure were measured on days 1 – 4 – 8 – 12 - 16 - 20 - 24 - 28 and 32 before and 6 h after treatment. At the end of the study, data from 216 eyes for each group were analyzed.

## Data analysis

In order to contrast normality, Shapiro Wilk was performed. Two-way ANOVA was performed and for the multiple comparisons of the results, Tukey and Sidak tests were performed using the Graphpad Prism<sup>®</sup> Version 6. Values of  $P < 0,05$  were considered statistically significant. Data were expressed as the median and interquartile range.

## Ethical statement

The Ethics Committee for Animal experimentation at Universidad de Antioquia on June 9, 2020 act number 133 approved all experiments in rabbits for this investigation (minimal risk).

## Results

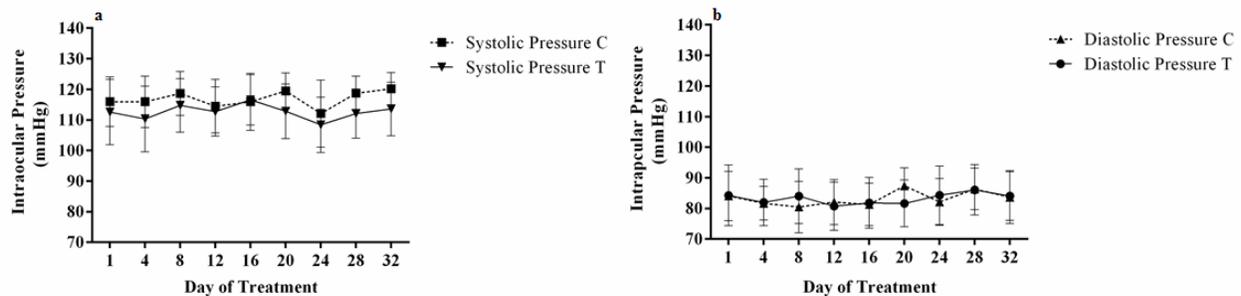
### Clinical signs

Before the start of the study, all the animals underwent general clinical and ophthalmological assessments, ruling out health problems in all individuals. All received placebo (C) and treatment (T) according to the experimental group to which they belonged, without any manifestation of clinical alteration or side effects, and they completed the study according to the schedule. Once the study was finished and it was verified that they were healthy, the rabbits were given up for adoption.

### Blood pressure

Both the control group (C) and the treated group (T) presented average normal values in both systolic (a) and diastolic (b) pressure without showing statistically significant differences between treatment days ([Figure 1](#)). However, a trend is noted where the group of animals

treated with amlodipine® (T) presents lower values than the control group, both in systolic and diastolic pressure.

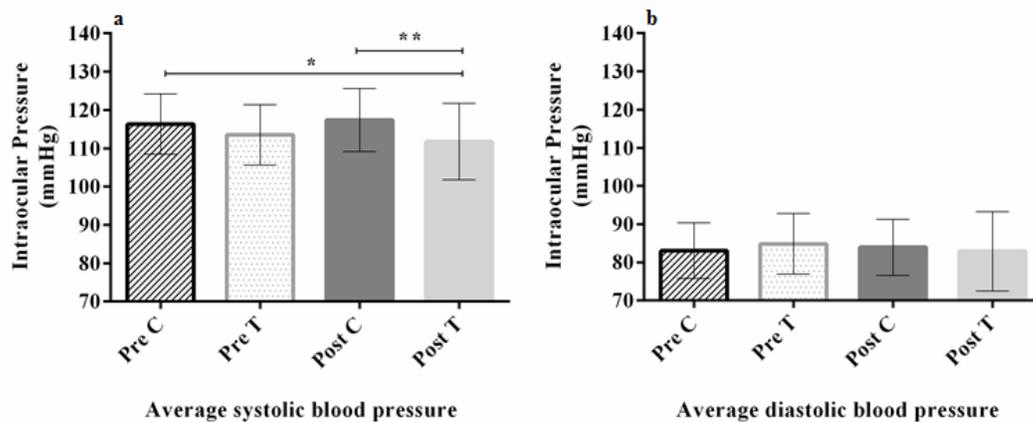


**Figure 1.** Average systolic and diastolic blood pressure. The figure shows the global average values of Systolic pressure <sup>(a)</sup> and Diastolic pressure <sup>(b)</sup> measured in millimeters of mercury (mmHg). Each point represents the mean value  $\pm$  S.E.M for each experimental group (n = 12) of control <sup>(C)</sup> group and treatment <sup>(T)</sup> group.

The systolic pressure was  $116.5 \pm 7.9$  mmHg and  $117.4 \pm 8.9$  mmHg, for experimental and control groups, respectively. The blood pressure before and after treatment in the treated was  $113.6 \pm 7.9$  mmHg and  $111.8 \pm 10.0$  mmHg (pretreatment and posttreatment respectively). No statistically significant differences were observed; nevertheless, when comparing the blood pressure before and after treatment between the control group and treated group differences were observed with  $p = 0.0314$  (pretreatment C vs T) and  $p = 0.0042$  (posttreatment C vs T). The control group showed a diastolic pressure of  $83.0 \pm 7.3$  mmHg (pretreatment) and  $83.9 \pm 7.4$  mmHg (posttreatment) while, the treated group had a diastolic pressure of  $84.9 \pm 8.0$  mmHg (pretreatment) and  $82.9 \pm 10.3$  mmHg (posttreatment). No statistically significant differences were observed between control and treated groups neither pre and posttreatment (Figure 2).

## Tonometry

In the first instance, the TONOVET Plus® was used, which is a rebound tonometer, starting with the left eye and then the right, then the measurement was taken with the TONO-PEN VET®, which is of flattening, in the same way starting with the left eye and then the right eye (after instillation of topical anesthetic) (Figure 3).



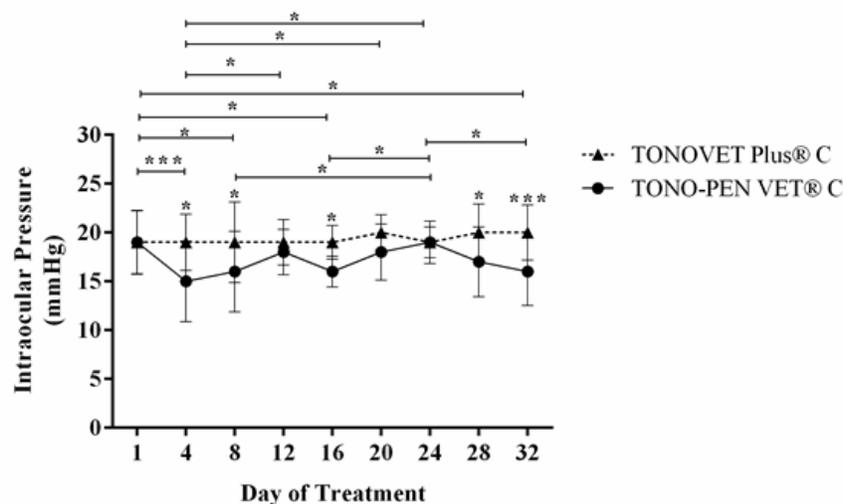
**Figure 2.** Pretreatment and posttreatment global average systolic and diastolic blood pressure. The figure shows the global average systolic <sup>(a)</sup> and diastolic <sup>(b)</sup> blood pressure measured before treatment <sup>(Pre)</sup> and six hours after treatment <sup>(Post)</sup> in control <sup>(C)</sup> and treatment <sup>(T)</sup> group. Each point represents the mean value  $\pm$  S.E.M for each experimental group (n = 12) of the Pretreatment C group. \* Pretreatment C vs Posttreatment T, ( $p$ -value=0.0314). \*\* Posttreatment C vs Posttreatment T, ( $p$ -value = 0.0042).



**Figure 3.** Rebound (TONOVET Plus<sup>®</sup>) and applanation (TONO-PEN VET<sup>®</sup>) tonometers. The image on the left shows the TONOVET Plus<sup>®</sup> tonometer and the image on the right shows the TONO-PEN VET<sup>®</sup> tonometer, both of them about to perform measurement intraocular pressure in New Zealand rabbits.

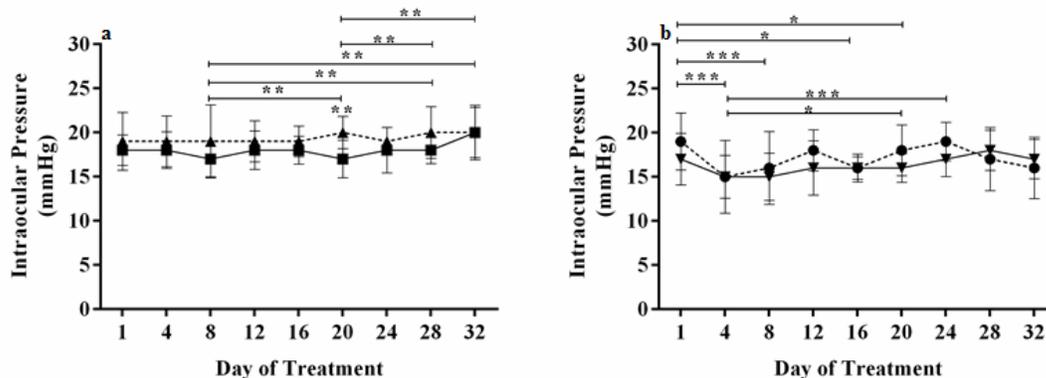
In the control group, no significant differences were observed between measurements before treatment and 6 h after treatment for each tonometer. The TONOVET Plus<sup>®</sup> did not present statistically significant differences between days of follow-up. The TONO-PEN VET<sup>®</sup> for its part

presented statistically significant differences between day 1 ( $19 \pm 3$  mmHg) and days 4 ( $19 \pm 3$  mmHg vs  $15 \pm 4$  mmHg), 8 ( $19 \pm 3$  mmHg vs  $16 \pm 4$  mmHg), 16 ( $19 \pm 3$  mmHg vs  $16 \pm 2$  mmHg) and 32 ( $19 \pm 3$  mmHg vs  $16 \pm 3$  mmHg); similarly, there were differences between days 4 ( $15 \pm 4$  mmHg) and days 12 ( $15 \pm 4$  mmHg vs  $18 \pm 2$  mmHg), 20 ( $15 \pm 4$  mmHg vs  $18 \pm 3$  mmHg) and 24 ( $15 \pm 3$  mmHg) and 24 ( $15 \pm 4$  mmHg vs  $19 \pm 2$  mmHg). Likewise, between days 8 and 24 ( $16 \pm 4$  mmHg vs  $19 \pm 2$  mmHg), 16 and 24 ( $16 \pm 2$  mmHg vs  $19 \pm 2$  mmHg) and days 24 and 32 ( $19 \pm 2$  mmHg vs  $16 \pm 3$  mmHg). Alternatively, statistically significant differences were found between TONOVET Plus® and TONO-PEN VET® on days 4 ( $19 \pm 3$  mmHg vs  $15 \pm 4$  mmHg), 8 ( $19 \pm 4$  mmHg vs  $16 \pm 4$  mmHg), 16 ( $19 \pm 2$  mmHg vs  $16 \pm 2$  mmHg), 28 ( $20 \pm 3$  mmHg vs  $17 \pm 4$  mmHg) and 32 ( $20 \pm 3$  mmHg vs  $16 \pm 3$  mmHg). When comparing the results obtained in the measurements of the control group, we can observe in a general way that the values obtained using the TONOVET Plus® ( $19 \pm 3$  mmHg, n = 216 eyes) are on average higher than the values obtained using the TONO-PEN VET® ( $17 \pm 3$  mmHg, n = 216 eyes) (Figure 4).



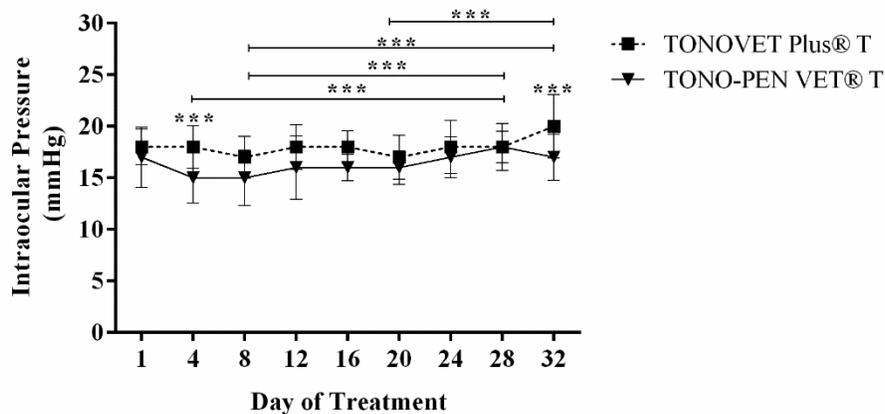
**Figure 4.** Control group tonometry measured using a rebound (TONOVET Plus®) and applanation (TONO-PEN VET®) tonometers. The figure shows the measures in millimeters of mercury (mmHg) of the intraocular pressure in the control group obtained using two tonometers. Each point represents the mean value  $\pm$  S.E.M for the control group h(n = 12) measured using the TON.

When comparing the control group with the treatment group for both TONOVET Plus® and TONO-PEN VET® (Figure 5), we can see that TONOVET Plus® (a) could identify statistically significant differences on day 20 of follow-up ( $20 \pm 2$  mmHg vs  $17 \pm 2$  mmHg) between the control group and the treatment group, respectively. Alternatively, between day 8 of the treatment group and days 20 ( $17 \pm 2$  mmHg vs  $20 \pm 2$  mmHg), 28 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg) and 32 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg) from the control group. Similarly, between day 20 of the treatment group and days 28 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg) and 32 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg) of the control group; For its part, the TONO-PEN VET® (b) indicated statistically significant differences between day 1 of the control group and days 4 ( $19 \pm 3$  mmHg vs  $15 \pm 2$  mmHg), 8 ( $19 \pm 3$  mmHg vs  $15 \pm 3$  mmHg), 16 ( $19 \pm 3$  mmHg vs  $16 \pm 1$  mmHg) and 20 ( $19 \pm 3$  mmHg vs  $16 \pm 2$  mmHg) of the treatment group. Likewise, between day 4 of the treatment group and days 20 ( $15 \pm 2$  mmHg vs  $18 \pm 3$  mmHg), 24 ( $15 \pm 2$  mmHg vs  $19 \pm 2$  mmHg) of the control group. In both tonometers, it was possible to show the trend to lower intraocular pressure values in the group treated with amlodipine® than those of the control group (TONOVET Plus® control ( $19 \pm 3$  mmHg,  $n = 216$ ) vs TONOVET Plus® treatment ( $18 \pm 2$  mmHg,  $n = 216$ ); TONO-PEN VET® control ( $17 \pm 3$  mmHg,  $n = 216$ ) vs TONO-PEN VET® treatment ( $16 \pm 2$  mmHg,  $n = 216$ )).



**Figure 5.** Intraocular pressure comparison between control and treatment groups measured using a rebound (TONOVET Plus®) and applanation (TONO-PEN VET®) tonometers. The figure shows the average of intraocular pressure comparing the control and treatment groups measured using TONOVET Plus® (a) and TONO-PEN VET® (b). Each point represents the mean value  $\pm$  S.E.M for each experimental group ( $n = 12$ ) of TONOVET Plus® a control group, TONOVET Plus® treatment group, TONO-PEN VET® control group, TONO-PEN VET® treatment group. (\* $p$ -value=0.0238), (\*\* $p$ -value=0.0036) (\*\* $p$ -value=0.0001).

When comparing the measurements obtained in the group of individuals treated with amlodipine<sup>®</sup> with both tonometers (Figure 6). TONOVET Plus<sup>®</sup> and TONO-PEN VET<sup>®</sup>, statistically significant differences between both tonometers can be observed on day 4 ( $18 \pm 2$  mmHg vs  $15 \pm 2$  mmHg) and 32 ( $20 \pm 3$  mmHg vs  $17 \pm 2$  mmHg). El TONO-PEN VET<sup>®</sup> showed statistically significant differences between days 4 and 28 ( $15 \pm 2$  mmHg vs  $18 \pm 2$  mmHg) and days 8 and 28 ( $15 \pm 3$  mmHg vs  $18 \pm 2$  mmHg). TONOVET Plus<sup>®</sup> indicated differences between days 8 and 32 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg) and days 20 and 32 ( $17 \pm 2$  mmHg vs  $20 \pm 3$  mmHg).



**Figure 6.** Intraocular pressure comparison between the treatment group measured with a rebound (TONOVET Plus<sup>®</sup>) and applanation (TONO-PEN VET<sup>®</sup>) tonometers. The figure shows the average of intraocular pressure between the treatment groups measured with TONOVET Plus<sup>®</sup> and TONO-PEN VET<sup>®</sup>. Each point represents the mean value  $\pm$  S.E.M for each experimental group ( $n = 12$ ) of the TONOVET Plus<sup>®</sup> treatment group. TONO-PEN VET<sup>®</sup> treatment group. (\*\*\*)  $p$ -value=0.0007).

## Discussion

Animal models have allowed great advances in different aspects of both human and veterinary medicine. Rabbits, specifically, have played an important role in research processes, both for developing surgical techniques, and in the evaluation and monitoring of new therapeutic options. At the ophthalmology level, they have also made it possible to understand the pathophysiology of such dire diseases as glaucoma<sup>(8,16,32)</sup>.

Systemic blood pressure can affect the structure and function of the eye as has been reported in different studies indicating that it has an influence on intraocular pressure, becoming a risk factor for the development and progression of glaucoma<sup>(2,6,17)</sup>. In these studies it has been indicated that not only hypertension, but also hypotension are risk factors for the development of glaucoma, since it can generate a reduction in the perfusion pressure of the central retinal artery and the posterior portion of the optic nerve (produced by cardiovascular changes that increase resistance to blood flow and / or reduce perfusion)<sup>(5,17)</sup>. On the other hand, it has been reported that the systemic arterial pressure fluctuates in relation to the circadian rhythm and in the hours of the night it can appear in normotensive and hypertensive patients, a reduction between 10% and 20% of the systemic arterial pressure while they sleep; This is due to a reduction in the activity of the sympathetic nervous system, to then return to its basal values early in the morning<sup>(6,31)</sup>. A study in healthy rabbits where intraocular pressure was monitored at different times of the day indicated that it is higher at 6 am than in the following hours of the day<sup>(30)</sup>. For the above reason, in our study, the arterial and intraocular pressure measurements were performed after 7 am, so as not to interfere with the circadian rhythm of the rabbits.

Alternatively, amlodipine<sup>®</sup> is effective for treating many cardiovascular diseases, including high blood pressure. Due to the above, we wanted to evaluate its effect on systemic arterial pressure in healthy rabbits; being able to show a trend where the group of animals treated with amlodipine<sup>®</sup> presents normal values but lower than the control group, both in systolic and diastolic pressure. Statistical differences were evidenced between the control group and the post-treatment group with amlodipine<sup>®</sup>, and there were no statistically significant differences between pretreatment and 6 h post-treatment within each group. The latter could be explained by the pharmacodynamics of amlodipine<sup>®</sup>; after oral administration, the onset of the effect is gradual, reaching a peak plasma concentration at 6 or 8 h, gradually decreasing blood pressure, which tends to slowly return to its initial pressure after a dose, at 24 to 72 h, without severe effects on heart rate. In chronic oral treatments, where amlodipine<sup>®</sup> is administered once a day, it has been noted that blood pressure decreases from the start of treatment showing little fluctuation during the dose interval. If the treatment was suspended after several days of supply, the blood pressure would gradually return to its initial value 7 to 10 days later, without the rebound effect. Given these characteristics, this drug has been used as a complement in the therapy of ocular pathologies such as glaucoma<sup>(1,11,22)</sup>.

Alternatively, the intraocular pressure (IOP) represents the primary risk factor for developing glaucoma; if a reduction in intraocular pressure is achieved, the incidence and progression of glaucoma can be controlled; in contrast, an increase in IOP can cause microangiopathies and a

reduction in ocular blood flow <sup>(13,19,31)</sup>. Therefore, within the ophthalmological evaluation, tonometry is a highly valuable diagnostic aid, allowing the identification of intraocular pressure alterations. Rebound tonometry (TONOVET Plus<sup>®</sup>) measures intraocular pressure by quantifying the de-acceleration of the probe after impact with the corneal surface while the applanation tonometer (TONO-PEN VET<sup>®</sup>) quantifies the force required to flatten a certain area of the cornea. Both types of tonometry can be affected by corneal thickness, as well as ulcerative, pigmented or edematous affectations <sup>(7,12,21)</sup>, therefore, this study was aimed to compare TONOVET Plus<sup>®</sup> and TONO-PEN VET<sup>®</sup> in healthy animals and to demonstrate their ability to detect changes in intraocular pressure under the effect of amlodipine<sup>®</sup>, a drug that regulates systemic arterial pressure. Intraocular pressure was measured for each rabbit with both tonometers, however, measurements were routinely started with the TONOVET Plus<sup>®</sup> and then with the TONO-PEN VET<sup>®</sup> because reports indicate that the first one may be affected by the application of topical anesthetics, which must perform applanation tonometry with the TONO-PEN VET<sup>®</sup>. Some of these reports even indicate a reduction in intraocular pressure immediately after the application of topical anesthetics with a possible duration of effect of up to 20 minutes <sup>(27)</sup>. There have been several possible explanations given to explain this phenomenon; within them are the relaxation of the pressure exerted by the eyelids on the eyeball, corneal relaxation, the eyeball and ciliary muscles; understanding that corneal-scleral stiffness and extra-ocular muscle tone can affect intraocular pressure <sup>(4,14,18)</sup>.

We observed that the control group, who received only water and the rabbits were adapted to the same management protocol as the animals treated with amlodipine<sup>®</sup>, did not present significant differences in intraocular pressure measurements before treatment and 6 h posttreatment with neither of the two tonometers. Alternatively, the TONE-PEN VET<sup>®</sup> of the control group showed greater variability between days of follow-up (significant statistical differences between day 1 and days 8, 16 and 32; on days 4 and days 12, 20 and 24; likewise, between days 8 and 24, 16 and 24, 24 and 32); while the TONOVET Plus<sup>®</sup> of the control group did not present statistically significant differences between days of follow-up. When comparing both tonometers, significant differences were found on days 4, 8, 16, 28 and 32, possibly due to the greater variability of the data obtained using the TONO-PEN VET<sup>®</sup>, we were able to observe that when measuring intraocular pressure in the control group, the values obtained in the same individuals with TONOVET Plus<sup>®</sup> (19 mmHg ± 3, n = 216 eyes) are on average higher than those obtained using TONO- PEN VET<sup>®</sup> (17 mmHg ± 3, n = 216 eyes). A similar trend was observed in the group treated with amlodipine<sup>®</sup> where the TONOVET Plus<sup>®</sup> indicated an average intraocular pressure of 18 mmHg ± 2 (n = 216 eyes) and the PEN-TONE VET<sup>®</sup> of 16 mmHg ± 2 (n = 216 eyes).

This phenomenon has been documented in other studies where a difference has been found in both tonometers between approximately 2 and 3 mmHg<sup>(18,30)</sup>. As we mentioned previously, it cannot be ruled out that this difference is partly caused by the effects of topical anesthetic that must conduct the measurement using the applanation tonometer; In some sensitive patients, it has been possible to identify reductions in intraocular pressure after the application of the topical anesthetic of up to 6-8 mmHg<sup>(23)</sup>, suggesting that the need for the use of topical anesthetic induces an underestimation of intraocular pressure.

Both TONOVET Plus<sup>®</sup> and TONO-PEN Vet<sup>®</sup> showed a lower intraocular pressure tendency in rabbits treated with amlodipine<sup>®</sup> (who also showed a lower systemic arterial pressure tendency than the control group), validating the fact that intraocular pressure is influenced by systemic arterial pressure. Additionally, the results also validate that dihydropyridine calcium antagonists, L-type selective for vascular smooth muscle such as amlodipine<sup>®</sup>, have great potential as supportive therapy for patients with glaucoma secondary to systemic hypertension.

## Conclusions

It was possible to validate the relationship between systemic arterial pressure and intraocular pressure in white New Zealand rabbits, reaffirming the use of rabbits as an animal model for the study of some ophthalmologic alterations. It was also possible to demonstrate the effect of amlodipine on systemic arterial pressure and its subsequent influence on intraocular pressure. Both rebound tonometry and applanation tonometry allowed measuring normal values of intraocular pressure in healthy rabbits; in addition, they were able to detect sensitive changes in intraocular pressure after amlodipine administration; however, rebound tonometry showed less variability in the results than applanation tonometry.

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## Conflict of Interest

None of the authors has a financial or personal relationship with other people or organizations that could improperly influence the design of the study, its results, or the writing and publication of this paper.

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