The Basic Values of Intraocular Pressure Measured by Rebound Tonometry in Guinea Pig

Diana-Larisa ANCUȚA1,2*, Diana Mihaela ALEXANDRU1, Maria CRIVINEANU1, Răzvan BOTEZATU3, Cristin COMAN2

1 Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine, Splaiul Independenței 105, Bucharest, Romania
2 "Cantacuzino" National Medical-Military Development Research Institute, Splaiul Independenței 103, Bucharest, Romania
3 Centrovet Veterinary Clinic, 52 – 54 Pascal Aristide sect. 3, Bucharest, Romania

* Corresponding author: D.L. Ancuța e-mail: diana.larisa.ancuta@gmail.com

Abstract
Elevated intraocular pressure (IOP) is the most important risk factor associated with the progression of glaucoma and the research on animal models is constantly evolving. Rebound tonometry has been shown to be useful for the evaluation of IOP, not only for the human patient but also for the animal patient. The aim of the paper is to assess intraocular pressure in guinea pigs in order to establish reference values. IOP was measured for three days, at different time intervals, in 40 guinea pigs, using the iCare Tonovet TV01 tonometer. The mean values obtained in coloured guinea pigs were 13.82 mmHg ± 1.64 mmHg in the right eye, 14.11 mmHg ± 1.68 mmHg in the left eye, and in white guinea pigs 12.56 mmHg ± 0.95 mmHg in the right eye, 13.02 mmHg ± 1.29 mmHg for the left eye. The values obtained from this study it can be concluded that the strain of coloured, records the highest values in the morning, which remain linear during the day, and decrease in the evening compared to the white strain which has a constant evolution during the day, and in the evening increases.

Keywords: Guinea pigs; intraocular pressure; rebound tonometry; Tonovet.

INTRODUCTION
The dynamic balance between the contents of the eye and the elasticity of its walls is given by intraocular tension and any abnormality in the drainage system can result in glaucoma (Durairaj et al., 2014) which is a chronic, multifactorial, asymmetric ocular disease, characterized by loss of retinal ganglion fibers, followed by damage to the optic nerve, narrowing of the visual field and finally, blindness (Panarelli et al., 2016). Among the factors that influence intraocular pressure is the thickness of the cornea, the thicker it is, the more it influences the tension in the ascending direction. Ocular hypertension can be an independent condition, encountered in the elderly and it is frequently associated with circulatory, cardiovascular or juvenile / congenital glaucoma in children (Feng et al., 2015).

Elevated intraocular pressure is the most important risk factor associated with the progression of glaucoma, therefore, it is necessary that patients from the early stages of the disease, constantly check the IOP values. As there is still no definitive cure for glaucoma, research on the association between IOP and disease progression continues today on animal models such as rabbit (Sarchahi and Bozorgi, 2012), monkey (Yang et al., 2015) or cat (Struebing and Geisert, 2015). Rebound tonometry has been shown to be useful for the evaluation of IOP, not only for human but also animals, the measurement procedure being easy, no anesthesia is required, the device is suitable for non-cooperating animals, it is portable and easy to handle. (Feng et al., 2015)

The guinea pig is a valuable model used in human vision research due to the ocular similarities with humans. This rodent is an important model for the study of eye structure (retina, cornea, lens) and eye diseases as well as cataracts, myopia, glaucoma
and uveitis (Seyed et al., 2016). Guinea pigs are diurnal rodents with a dichromatic view. Their retina is avascular, thinner than that of animals with vascular retina, and the blood is supplied exclusively by the choroidal circulation. In addition to the rods, the guinea pig retina includes sensitive cones at medium and short wavelengths, which occupy its upper and lower areas, while the transition area contains both cone types and cells with both pigments (Ostrin et al., 2014). The guinea pigs do not have fovea and yet the retinas provide a visual acuity of about 2.7 cyc/deg. The optic nerve contains a collagen lamina sieve with connective tissue bundles, the crystal lenses are relatively large and the vitreous chambers relatively small compared to primates (Troilo et al., 2019). Evaluation of intraocular pressure is a crucial step that completes the diagnosis of the aforementioned diseases. The guinea pig is a docile animal, easy to handle, with a fast growth rate and availability of reproduction in captivity (Yang et al., 2015; Teixeira et al., 2014; Deng et al., 2013; Gøtzsche, 2014). The fact that guinea pigs are born with open eyes, has the advantage that they can be introduced to studies from birth, contributing to the research of infantile eye abnormalities.

Therefore, the aim of the study was to evaluate IOP at different strains of guinea pigs, at different ages and by sex, precisely to find the most suitable guinea pig model for the study of conditions dependent on intraocular pressure. The results obtained should lead to the establishment of IOP benchmarks which is an important goal for further research and development of new treatments.

MATERIALS AND METHODS

The research has been carried out in the "Cantacuzino” National Medical-Military Development Research Institute (IC).

Ethical Statement

The animal study was approved by the Ethics Commission of the "Cantacuzino” National Medical-Military Development Research Institute and authorized by the competent authority. The procedures were carried out in accordance with the provisions of Directive 2010/63/EU on compliance with the rules for the care, use and protection of animals used for scientific purposes.

Animals

The studied population consisted of 40 healthy guinea pigs, different strains (tricolor and white), males and females, youth and adults. The white guinea pig is the most used in biomedical research, of all the existing strains (Figure 1). The hair is short, and the color of the robe is determined by the genotype of the albino locus which is composed of four alleles. The most important is the albino allele, which results in different degrees of dilution / discoloration of the skin, hair and eyes (Seeman and Jennifer, 2016). The genotype of tricolor guinea pigs (Figure 2), in addition to the albino locus, also contains the agouti locus (responsible for the production of black) and the brown locus (that changes black to different shades of brown).

![Figure 1. Guinea pig, Dunkin Hartley/white strain](image1)

![Figure 2. Guinea pig, tricolor strain](image2)

The average weight for young females was 250 grams ± 30 grams, and that of young males 300 grams ± 50 grams. At the start of the study, adult males weighed 600 grams ± 50 grams and adult females weighed 500 grams ± 50 grams. The animals come from the colonies raised within the Băneasa Animal Facility (SB) of the "Cantacuzino" National Medical-Military Development Research Institute (IC), a unit authorized for the breeding and use of animals for scientific purposes. They were acclimatized 5 days before the first test, the shelter was made in cages of 5 animals (612 x435 x216 mm), labeled, placed in the experimental space of SB, at a temperature of 22 - 24°C and relative humidity of 45-65%, with cycles of 12h light and 12 h dark (the light program being between 7oo-19oo). Both during the acclimatization and in the testing period, the animals received food (granulated combined fodder) and water ad libitum (Table 1).
IOP measurement with the iCare Tonovet rebound tonometer

Rebound tonometry is a non-invasive method of measuring IOP that uses a magnetic probe propelled toward the center of the cornea by a solenoid. The IOP value is correlated with the deceleration of the probe at the moment of impact so that an eyeball with low IOP will have a longer impact time and slow deceleration speed, and an eyeball with increased IOP will have a short impact with fast deceleration.

The iCare Tonovet tonometer has 3 operating modes: "h", "d" and "p" for horses, dogs / cats and other species, respectively. The device was used according to the manufacturer's instructions, keeping the magnetic probe in a horizontal position and the tip of the tonometer directed perpendicular to the center of the cornea. The equipment was set to calculate the average of 6 consecutive IOP measurements for each eye.

At the beginning of the experiment all animals were subjected to normal physical and ophthalmological examinations, and the IOP evaluation procedure was performed by setting the device to the "p" operating mode. Only one examiner performed the measurements at different times of the day, namely at 6 am, 12 pm, 6 pm and 9 pm, for 3 days. One week after the end of the study, all animals were evaluated and no signs of conjunctivitis, blepharitis, corneal ulceration or other eye complications were observed.

Statistical analysis

Statistical analysis was performed using the current version of Microsoft Excel. The results presented represent the mean values ± SD. The paired T-test was used to compare the difference between two eyes in an animal at a certain time of day, and T <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

The mean IOP in the 15 hours examination of all guinea pigs (80 eyes) was 13.38 mmHg ± 0.23 mmHg, indicating a normal distribution. A statistically significant difference between the right and left eye could be observed only in white guinea pigs, where T =0.03, at 18 pm and in tricolor guinea pigs, at 21 pm, when T = 0.04. (T<0.05). (Table 2)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Calculated values</th>
<th>6 am</th>
<th>12 pm</th>
<th>9 pm</th>
<th>6 am</th>
<th>12 pm</th>
<th>9 pm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average IOP</td>
<td>RE</td>
<td>LE</td>
<td>RE</td>
<td>LE</td>
<td>RE</td>
<td>LE</td>
</tr>
<tr>
<td></td>
<td>Maximum IOP</td>
<td>15.53</td>
<td>15.46</td>
<td>16</td>
<td>16.2</td>
<td>16.06</td>
<td>15.86</td>
</tr>
<tr>
<td></td>
<td>Minimum IOP</td>
<td>12.55</td>
<td>12.18</td>
<td>12.33</td>
<td>11.86</td>
<td>12.53</td>
<td>12.33</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>1.18</td>
<td>1.31</td>
<td>1.37</td>
<td>1.84</td>
<td>1.68</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>T-test</td>
<td>0.37</td>
<td>0.32</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>White guinea pig</td>
<td>13.98</td>
<td>13.08</td>
<td>12</td>
<td>12.15</td>
<td>12.9</td>
<td>13.41</td>
<td>12.36</td>
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<tr>
<td></td>
<td>Maximum IOP</td>
<td>13.66</td>
<td>14.46</td>
<td>13.2</td>
<td>13.4</td>
<td>13.93</td>
<td>14.86</td>
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<tr>
<td></td>
<td>Minimum IOP</td>
<td>12.6</td>
<td>12.26</td>
<td>10.2</td>
<td>10.86</td>
<td>11.93</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>0.40</td>
<td>0.83</td>
<td>1.18</td>
<td>1.21</td>
<td>0.80</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>T-test</td>
<td>0.38</td>
<td>0.31</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
In fact, the data obtained during the IOP evaluation, in tricolor guinea pig, indicated maximum values in the morning, with an increasing tendency between 12-18 pm, then with a downward evolution in the evening. The tricolor youth registered values between 9.2-14.6 mmHg and the tricolor adults, higher values: 12.2-17.2 mmHg (Figure 3).

**Figure 3:** Maximum (left) and minimum (right) values of IOP in tricolor guinea pigs (abbreviations: TY = tricolor youth, TA= tricolor adults)

The mean IOP value in white guinea pigs during the study was 12.78 mmHg, with limits ranging from 9.2 to 14.25 mmHg in young and 10.4 to 16 mmHg in adults (Figure 4).

**Figure 4:** Maximum (left) and minimum (right) values of IOP in white guinea pigs (abbreviations: WY = white youth, WA= white adults)

The research proved that it is possible to measure IOP using the rebound tonometry method at guinea pigs, they were not anesthetized, and the procedures was performed easily, without causing eye damage. The results showed that the values obtained at each examination, regardless of time, sex, age or line, are maintained in the normal parameters. Comparing the youth with the adults at the studied strains of guinea pigs, it can be seen that tricolored adults (males and females) register higher IOP values during the day, decreasing in the evening. The white strain provided consistent results with values that are lower in the morning and higher in the evening. In the tricolored youth, there was a tendency to increase the IOP in the evening, especially in males, in contrast to the youth in the white strain whose IOP decreases (Figure 5 and figure 6).

**Figure 5.** IOP mean values in tricolor / white guinea pigs, male / female, adults / young (abbreviations: RE - right eye, LE – left eye, TFY - tricolor female youth, WFY - white female youth, TMY - tricolor male youth, WMY - white male youth, TFA - tricolor female adult, TMA - tricolor male adult, WFA - white female adult, WMA - white male adult)
Using rebound tonometry, the mean IOP values obtained from tricolor and white guinea pigs were lower than those recorded by application tonometry which reported an average of 18.27 ± 4.55 mm Hg (Coster et al., 2008). Compared to other animals, such as rabbit (15.21 ± 1.0 mmHg), chinchilla (17.71 ± 4.17 mmHg) (Lima et al., 2010), Wistar rat (18.4 ± 0.1 mmHg) and with mice DBA / 21 (19.3 ± 0.4 mmHg), BALB / c (10.6 ± 0.6 mmHg), C57 - BL / 6 (13.3 ± 0.3 mmHg) and 16.4 ± 0.3 mmHg (CBA) can be said that the mean value of IOP in guinea pigs is close to that of mice (Wang et al., 2005). To accurately measure IOP, it is necessary to use reliable instruments, the Goldmann application tonometer (GAT) being the gold standard for IOP measurements (Shu et al., 2019). Because rebound tonometry makes very short contact with the surface of the cornea, the need for topical anesthesia is eliminated, this method is currently preferred because the disadvantages of using GAT include the need for local anesthesia, a slit lamp and staff experienced in using a tonometer. The method cannot be applied to bedridden people or people with corneal abnormalities and it is also necessary to disinfect the probe between patients. Even if the values expressed are different from those given by application tonometry, rebound tonometry provides reliable and accurate data to humans (Abraham et al., 2008), animals and birds of prey (Reuter et al., 2010).

The research results showed that the average IOP in guinea pigs is 13.38 mmHg ±0.23, in tricolors is 13.96 mmHg, and in white guinea pigs is 12.78 mmHg, values much lower than those reported by Coster et al. (18.27 mmHg ± 4.55) whose evaluation was based on the application tonometry, thus suggesting that the chosen IOP measurement method may influence its values (Coster et al., 2008), in the case of rebound tonometry IOP being much smaller. Studies in mice (Garcia-Resua et al., 2006) have also shown a mismatch between IOP values obtained by the two types of tonometry (Nakamura et al., 2006; Kim et al., 2007). In the case of our study, the IOP fluctuations in tricolor and white guinea pigs were smaller than those observed in humans, this aspect can be explained by the fact that the corneal thickness in guinea pigs is 227.85 ± 14.09 um, and that of humans of is 500 um, results obtained by ultrasound pachymetry in both cases (Rao et al., 2014; Cafaro et al., 2009).

CONCLUSIONS

Based on the experiment, we can suggest that the IOP reference values in young tricolor guinea pigs are between 9.2-14.6 mmHg mmHg, in young white guinea pigs between 9.2-14.25 mmHg, in tricolor adults between 12.2 – 17.2 mmHg and in white adult guinea pigs between 10.4 - 16 mmHg. All this information can be useful for the study of human eye diseases, because IOP in guinea pigs is similar to that of humans, this rodent is a good experimental model for developing new treatments and also the values expressed can be useful to the examiner when we talk about guinea pigs as pets.

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REFERENCES


Conflicts of Interest
The authors declare that they do not have any conflict of interest.