NONINVASIVE MEASUREMENT OF INTRAOCULAR PRESSURE IN RATS WITH THE ICARE TONOVET REBOUND TONOMETER

Diana – Larisa ANCUȚA[6,2], Fabiola IONIȚĂ [1,2], Diana ALEXANDRU[7], Maria CRIVINEANU [2], Cristin COMAN[1,3,4]

e-mail: diana.larisa.ancuta@gmail.com

Abstract

Rebound tonometry is an accurate method of measuring intraocular pressure (IOP) that is easy to perform and does not involve the use of anesthesia. This, together with biological and genetic advances contributes to the use of ocular etiopathogenetic data of rats. The aim of the study was to evaluate the applicability, reproducibility and accuracy of a rebound tonometer in measuring IOP in rats. IOP was measured three times, at different time intervals, in 40 male and female Sprague Dawley rats, 10 weeks old. The animals studied were kept in normal living conditions, not subject to external stress. The mean IOP expressed consecutively of six successive measurements for each eye was determined with the iCare TonoVet rebound tonometer. The readings generated gave IOP values between 13.3 - 14.5 mmHg in males and 12.6-16.1 mmHg in females. There were no significant differences between the eyes in terms of the values obtained in a measurement so the variability was 0.02 mmHg. Rebound tonometry is convenient, can be used without topical anesthesia and provides fast and accurate results. These can be useful to the clinician, when we talk about the rat as a pet or to scientists, when the rat is chosen as an animal model for various biomedical research.

Key words: rat, iCare tonovet, rebound tonometry

In experimental research involving the eye, it is desirable to use animal models that are easy to handle and less expensive such as rats. The physiology and pathophysiology of the rat has already been studied, at present there is enough information worthy of consideration when studying optic nerve damage, ocular inflammation or glaucoma.

Any animal model for glaucoma research, for example, requires accurate and reproducible measurements of intraocular pressure (IOP) which is an accepted risk factor for this condition (Mermoud et al., 1994). Lately, the animal models preferred by ophthalmologists, are rodents

From an anatomical point of view, the rat's eye presents the head of the optic nerve obstructed by a series of arteries and veins arranged in the form of a disc that enters the lower neural portion of the optic nerve head. This can be considered a significant difference from the anatomy of the human eye, where blood vessels are already inside the optic nerve, at the entrance to the eyeball. Histologically, in cross-section, the neuronal portion of the head of the optical rat nerve has an oval shape at the level of the Bruch membrane and sclera, with the short axis oriented vertically (Morrison et al., 2015).

Another important feature of the optic nerve head of the rat is the absence of the collagen cribrosa lamina, in rodents, being replaced by "glial lamina" (Sun et al., 2009) which is made up of astrocytes oriented over the scleral canal and perpendicular to the axonic fascicles (Tehrani et al., 2014). Thus, the glial cribrosa lamina of the rat contains numerous relationships that will help us use these animals to understand how the cellular biology of the optic nerve head responds to increases and fluctuations in IOP and affects axonal lesions in human glaucoma (Burgoyne C.F., 2011).

The blood supply to the head of the optic nerve of the rat is made through the ophthalmic artery, as in primates that immediately under the optic nerve trifurcate, in two long posterior ciliary arteries and the central retinal artery. The latter, entering the lower globe of the optic nerve, provides capillary beds of the retinal nerve fiber layer and the anterior portion of the head of the optic nerve. All these capillary beds either pass into the central vein of the retina or into the veins that are in the sheath of the optic nerve. The veins of the optic nerve sheath also communicate with the

[1] Cantacuzino National Medico-Military Institute for Research and Development, Bucharest
[2] University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Bucharest
[3]Spiru Haret University, Faculty of Veterinary Medicine, Bucharest
[4]The Fundeni Clinical Institute, Center of Excellence in Translational Medicine, Bucharest

central retinal vein and choroid veins through a large peripapillary sinus. The presence of this communication highlights the importance of not disrupting any aspect of venous outflow when modeling increased IOP. (Morrison et al., 2005).

Another important element on which IOP depends is the aqueous humor. In the rat, as in primacy, the conveyance of aqueous humor occurs through the trabecular network, in the Schlemm canal and through the limbal sclera through the collecting channels to the episcleral venous circulation (Morrison et al., 1995) This episcleral plexus, as well as the Schlemm canal is continuous.

In the last decade, the most popular method of evaluating IOP is rebound tonometry, even if the tonometry performed by contact methods is accurate and repeatable. The important landmark for which the rebound tonometry is preferred is the facility of handling the device in new pets, including laboratory animals. In these, the use of corneal anesthesia is not necessarily necessary when IOP is measured by rebound tonometry. I Care Tonovet is a portable, easy-to-use instrument that is equipped with a probe that requires very short contact with the corneal surface to give precise IOP results. One of the disadvantages of using the Tonovet is that the position of the probe must be a horizontal one at contact with the cornea, perpendicular to its surface, because if the wrong angle or the peripheral corneal region on which the probe is applied, it can provide erroneous values of the IOP (Rodrigues B et al. 2021).

The purpose of our study is to evaluate the applicability, reproducibility and accuracy of the rebound tonometer to healthy rats in the Sprague Dawley strain. The values obtained from the IOP evaluation are useful when the researches concern the prevention, diagnosis or treatment of eye diseases or for pet's clinician to diagnose and treat eye diseases.

MATERIAL AND METHOD

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.

The research has been carried out in the "Cantacuzino" National Medical-Military evelopment Research Institute (CI), Preclinical Testing Unit.

For the IOP measurement, 40 rats, the Sprague Dawley strain, males and females (20:20), aged 8-10 weeks, were used. The animals come from the Baneasa Animal Facility (BAF), an authorized CI for breeding and use of animals for scientific purposes. During the experiment, the animals were accommodated in individually ventilated cages, arranged in the BAF experimentation space where the ambient temperature was 22-24^{0}C, the relative humidity of 45-65%, and the light/dark cycles of 12/12h. Food and water were provided ad libitum.

The iCare Tonovet tonometer is a non-invasive IOP measurement tool that uses a probe that propagates perpendicular to the cornea using a solenoid. The device has 3 modes of operation "h", "d" and "p" for horses, dogs / cats and other species, respectively. In the case of our study it was set to "p" mode and was used according to the manufacturer's instructions. The equipment was set to calculate the average of 6 consecutive IOP measurements for each eye. At the beginning of the experiment, all the animals were examined ophthalmological, and a single examiner performed the workmanship for measuring the IOP. Every day 0, day 44 and day 70 of the experiment, the animals were handling by an operator, and the examiner, measured the IOP keeping the device pointing perpendicular to the cornea. The final IOP value indicated by the device represented the average of 6 consecutive measurements. The IOP measurement surgery was performed 3 times, at same times of the day, and at the end of the study, no animal showed corneal lesions or other conditions following the use of the Tonovet rebound tonometer.

Figure 1 : iCare Tonovet rebound Tonovet tonometer

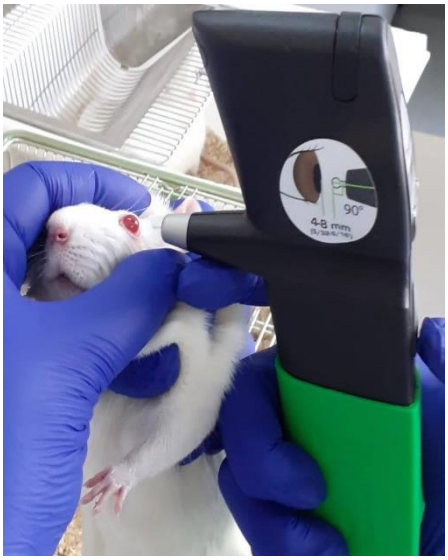

Figure 2: Measurement of IOP

RESULTS AND DISCUSSIONS

During the examinations, the animals were restraining manually and without anesthesia. All procedures were completed in less than 5 minutes per animal, at each measurement the same two people were used so that the animals are not subjected to stress.

Statistical analysis was performed using Microsoft Excel, the current version.

At the beginning of the study, animals showed PGI values between 13.2 and 13.6 mmHg in males and females. For the next two measurements, the recorded values were slightly increased but did not exceed 16.1 mmHg, which means that the reference values of the IOP in Sprague Dawley rats fall between 13.3 - 14.5 mmHg in males and 12.6-16.1 mmHg in females (table 1).

Tabel 1.

Average and standard deviation of IOP in rats

Sex	Day 0		Day 44		Day 77	
	LE	RE	LE	RE	LE	RE
Males	13,6 ±0,96	13,3 ±1,63	14,5 ±2,67	14,3 ±1,25	15,7 ±1,15	15,5 ±2,79
Females	13,2 ±1,81	13,6 ±2,83	16,1 ±2,37	12,6 ±2,17	15,7 ±1,15	15,5 ±2,79

No significant differences between the eyes (t ‹ 0,05) nor between the measurement intervals were observed (Figure 3). So, using the rebound tonometry method, we were able to prove that it was well tolerated by rats, with no eye injuries recorded. The technique did not involve anesthesia of the corneal surface, the same observations being recorded in previous studies on dogs, horses (Knollinger A.M, 2005), cats (Rusanen E, 2010), ruminants (Peche N., 2018), chinchillas (Snyder K.C., 2018) or humans (Pakrou N, 2008)

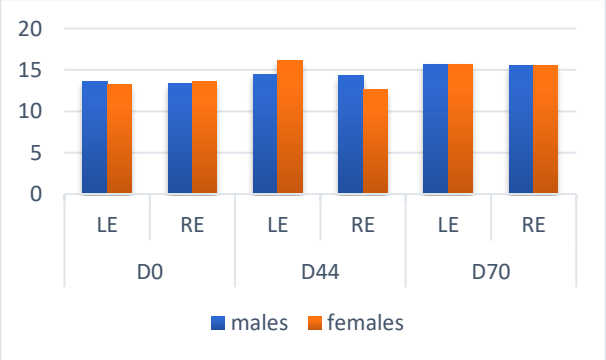

Figure3: IOP oscillations between the left and right eyes during the experiment

Albino rats are commonly used in the study of eye conditions such as glaucoma but also in general studies of non-clinical toxicity of chemicals and drugs. In particular, Sprague – Dawley rats are among the most widely used animal strains in many laboratories because they are suitable for toxicity assessment studies, with extensive biological reference data available. Observation of ocular toxicity may lead to the suspension of the further development of the compounds or to the regulation of the further use of the chemical in order to avoid human exposure. In non-clinical studies of in vivo toxicity, the eyes are typically evaluated by observing clinical signs and conducting ophthalmological and histopathological examination to assess the potential of a drug for inducing ocular toxicity (Morita J. et al., 2020). Morphological eye changes are mainly found through ophthalmological examination using techniques such as bio microscopy, tonometry and direct or indirect ophthalmoscopy (OECD guideline, 2018).

The results of our research on Sprague Dawley rats have highlighted values close to those obtained on Wistar rats (17.3 mmHg±5.25mmHg), which in turn are whitish and whose eyes are devoid of melanin pigments. However, there are studies that show that there are no considerable variations in IOP between the eyes of albino and pigmented rats, but rather, IOP seems to be dependent on the ciradian rhythm (Valiente-Soriano F.J et al., 2015), in albinos frequently registering ischemic lesions caused by the increase of IOP following prolonged exposure to light.

CONCLUSIONS

By measuring the IOP with the iCare Tonovet rebound tonometer, we obtained values similar to those reported in the specialized literature, which strengthens the credibility of the working method and technique. The data obtained in our experiment suggest that the limits of the IOP values in Sprague Dawley rats fall between 12.6 (±2.17) - 16.1(±2.37) mmHg. Information to be useful when this strain of rats is chosen for ophthalmological studies or even when choosing the rat as a pet.

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