• Basic Research •

Intraocular pressure with rebound tonometry and effects of topical intraocular pressure reducing medications in guinea pigs

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Abstract

• AIM: To investigate the intraocular pressure (IOP) of adult guinea pig eyes with rebound tonometry (RBT), and assess the effects of four distinctive topical IOP reducing medications including Carteolol, Brimonidine, Brinzolamide and Latanoprost.

• METHODS: The IOPs of twenty-four 12-week-old guinea pigs (48 eyes) were measured every two hours in one day with RBT as baselines. All the animals were then divided into four groups (Carteolol, Brimonidine, Brinzolamide and Latanaprost groups, *n*=6). The IOPs were measured and compared to the baseline 1, 2, 3, 5, 7, 9, 15 and 24h after treatment.

• RESULTS: The mean baseline IOP of 24 guinea pigs (48 eyes) was 10.3±0.36 mm Hg (6-13 mm Hg) and no binocular significant differences of IOPs were observed (*t*=1.76, *P*>0.05). No significant difference of IOP in Carteolol group at each time point was observed before and after treatment (*t*=1.48, *P*>0.05). In Brimonidine group, IOP was 2.2±1.9 mm Hg lower than the baseline after one hour (*t*=3.856, *P*=0.003) and lasted for one hour. In Brinzolamide group, IOP was 1.4±1.1 mm Hg lower than the baseline after one hour (*t*=4.53, *P*=0.001) and lasted for 7h and the IOP declined most at 3h. In Latanaprost group, IOP was 2.1±1.3 mm Hg lower than the baseline after one hour (*t*=6.11, *P*=0.001) and lasted for one hour.

• CONCLUSION: The IOP of guinea pig eyes is relatively stable compared to human eyes. In four reducing IOP medications, no significant effect of Carteolol is observed. Brinzolamide has the longest duration, while the Brimonidine has the shortest duration and the maximum level of treatment.

• KEYWORDS: guinea pig; intraocular pressure; Carteolol; Brinzolamide; Brimonidine; Latanoprost
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INTRODUCTION

Glaucoma is an optic neuropathy characterized by optic disk cupping and visual field loss[1]. The mechanism of raising intraocular pressure (IOP) in glaucoma is impaired resulting from abnormalities within the drainage system of the anterior chamber angle or impaired access of aqueous to the drainage system[2]. Treatment is directed toward reducing the IOP and correcting the underlying cause[3]. Until now, the experimental animals in glaucoma research are rabbit[4], monkey[5-6] and cat *etc*[7]. However, such animals all have the limitations of long growth cycle, difficult to acquire, or much expensive price. In recent years, the guinea pigs were introduced as a new animal model. Similar to the other rodents, guinea pigs owned the advantages including rapid growth, inexpensive price, breeding readily in captivity and being easily available[8-10]. However, the relationship between IOP and guinea pigs had not been adequately studied.

There is no known cure for glaucoma, but many forms of the disease are associated with pathological elevation of IOP, and elevated IOP is the most important risk factor associated with progression of the disease[11]. Tonometry had evolved greatly over decades. Some of which had proved useful for IOP assessment not only in human patients, but also in animal subjects for research purposes[11-12]. However, the tonometry which was used in humans is not appropriate to measure IOP like guinea pigs because the small eyeball size. The common tonometry which was used in rodent is Tonopen. Such an

instrument is sometimes difficult to measure and susceptible to be influenced by manipulator[13]. In contrast, the rebound tonometry (RBT) has the obvious advantage to measure the IOP of small animals[14-15]. The device is simple, easy to use and portable, does not require eye drops and is particularly suitable for children and non-cooperative patients[16].

Until now, there are four kinds of glaucoma therapy drops which are commonly used including beta-adrenergic blocking agents, carbonic anhydrase inhibitors, alpha-adrenergic agonist and prostaglandin analogs. The mechanisms of these four reducing IOP medications are quite different, and therefore, more work is needed to make clear the effect of topical reducing IOP eye drops in guinea pigs eyes.

Therefore, in the current research, RBT was used to obtain the baseline IOP of guinea pigs. The effects in small animal eyes of four topical reducing IOP eye drops were investigated. We hoped to be able to gain more knowledge of using guinea pigs in glaucoma research.

MATERIALS AND METHODS

Animals Twenty-four guinea pigs (English short hair stock, tricolor strain, 12 weeks of age) were obtained from the laboratory of Fudan University. The treatment and care of the animals were conducted according to the ARVO statement for the use of animals in Ophthalmic and Vision Research, and the animal research was approved by the Local Animal Care and Ethics Committee of Fudan University (Shanghai, China).

Experiment Instruments and Reagents RBT (SW-500), which determined IOP by bouncing a tiny plastic tipped metal probe against the cornea was used in the current research. The device used an induction coil to magnetise the probe and fired it against the cornea. As the probe bounced against the cornea and back into the device, it created an induction current from which the IOP was calculated.

Finnpipette (labsystems, Inc.), 2% Carteolol eye drops (Mikelan, C14200168681), 0.15% Brimonidine eye drops (Alphagan, C1420060292), 1% Brinzolamide eye drops (Azopt, 014200 220527), 0.005% Latanaprost eye drops (Xalatan, B01008361) were used in study.

Experimental Design All measurements were performed by one researcher who was masked to the identity of the experimental groups. The guinea pig was placed on the table and kept quiet. Three repeated measurements were performed in the horizontal meridian. Animal were anaesthetised with one drop of 0.5% proparacaine hydrochloride (Alcon).

Baseline Intraocular Pressure Measurement The IOPs of twenty-four guinea pigs (48 eyes) were measured every 2h at the beginning of 0:00, IOP of the whole days was recorded at each time point.

Intraocular Pressure Measurements of Four Treatment Groups The IOP of four treatment groups were measured one week after the baseline measurements. All the guinea pigs were divided into four groups (Carteolol, Brimonidine, Brinzolamide and Latanaprost groups, n=6). All the animals were given the respective treatment 50 μL every drop with Finnpipette. The IOPs were measured 1, 2, 3, 5, 7, 9, 15 and 24h after treatment. In order to compare to the baseline, all the guinea pigs were raised without any treatment for one week, and the baseline at each specified time points were measured again.

Statistical Analysis The raw data were processed using SPSS 20.0. The graph was created by Graphpad 5.0 software. The results were presented as means±standard deviation. Paired t-test was used to compare the difference between two eyes in one animal. The data at each time point were analyzed with analysis of variance (ANOVA). Paired t-test was also used to compare the difference before and after treatment in one animal. ANOVA was also applied to test the difference of IOP at each time point in one group. P<0.05 was considered significantly different.

RESULTS

Baseline Intraocular Pressure The mean twenty-four hours IOP of the whole 24 guinea pigs (48 eyes) was 10.3±0.36 mm Hg, which followed a normal distribution. No significant difference in IOP was found in 24 guinea pigs throughout 24h (ANOVA, F=3.49, P=0.76; Figure 1A). The minimum and maximum values in one day were 9.88±0.82 mm Hg and 10.73±1.05 mm Hg relatively. The amplitude of IOP was 0.85±1.32 mm Hg. Meanwhile, no binocular difference in IOP was observed at each time point (Figure 1B).

Intraocular Pressure of Four Treatment Groups In Carteolol group, the IOPs after treatment during 24h were shown in Figure 2. No significant differences were observed at each time point compared to the baseline IOPs.

In Brimonidine group, the IOPs after using Brimonidine eye drops during 24h were shown in Figure 3. Significant difference was observed after one hour (t=3.856, P=0.003), the IOP was 2.2±1.9 mm Hg lower than baseline and the difference lasted for two hours.

In Brinzolamide group, the IOPs after using eye drops during 24h were shown in Figure 4. Significant difference was found after one hour (t=4.53, P=0.001). The IOP after treatment was 1.4±1.1 mm Hg lower than baseline and the effect lasted for 7h. The decreased amplitude of IOP reached the maximum at 3h. The IOP was 2.0±2.29 mm Hg lower than baseline at 3h (t=3.02, P=0.012). Afterwards, the IOP increased. Significant difference was found at 24h (t=5.20, P=0.001). The IOP was 1.3±0.89 mm Hg higher than baseline IOP at the end of the experiment.

In Latanaprost group, the IOPs after taking the drug during 24h were shown in Figure 5. Significant difference was observed after one hour (t=6.11, P=0.001). The IOP was 2.1±1.3 mm Hg lower than baseline. The difference lasted for one hour.

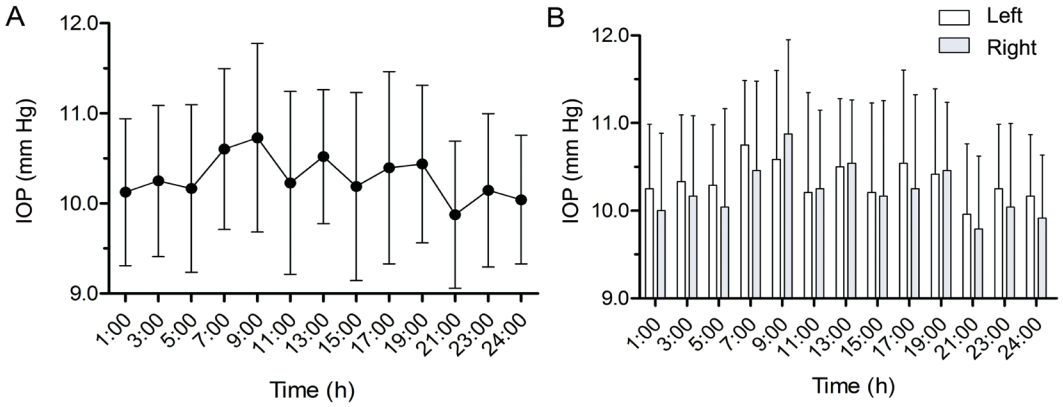

Figure 1 Baseline IOP A: The baseline IOPs of adult guinea pigs at each time point during 24h. The minimum and maximum IOP values in one day appear at 21:00 and 9:00 relatively. B: No binocular difference of IOPs was observed at each time point, $P>0.05$.

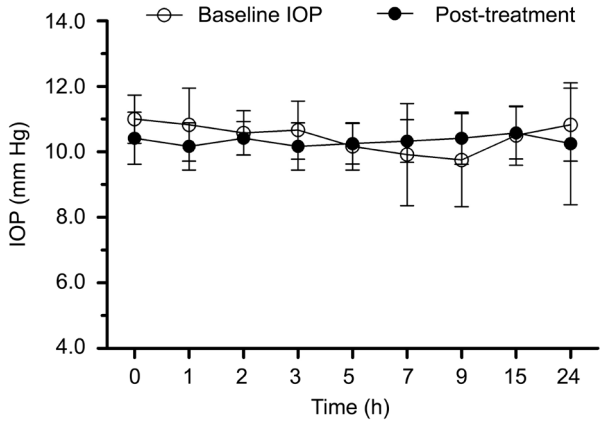

Figure 2 The IOPs before and after Carteolol treatment during 24h.

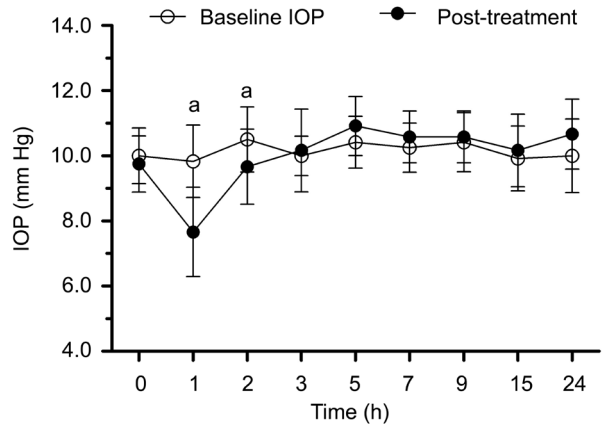

Figure 3 The IOPs before and after Brimonidine treatment during 24h $^{a}P<0.05$ between post-treatment IOP and baseline.

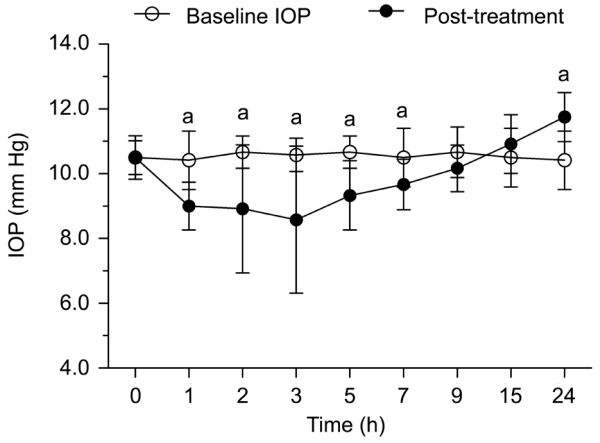

Figure 4 The IOPs before and after Brinzolamide treatment during 24h $^{a}P<0.05$ between post-treatment IOP and baseline.

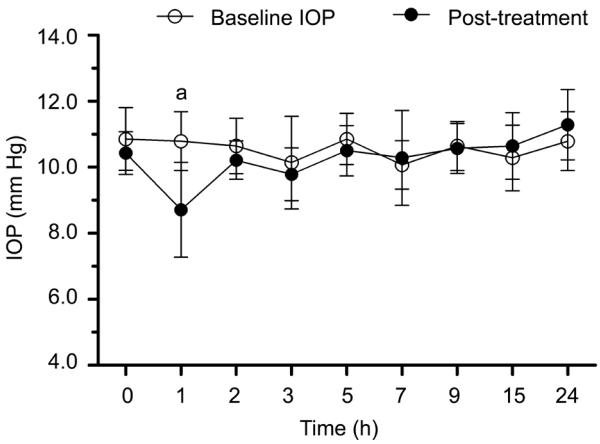

Figure 5 The IOPs before and after Latanaprost treatment during 24h $^{a}P<0.05$ between post-treatment IOP and baseline.

DISCUSSION

Our current research proved it was feasible to use RBT to measure the IOP of guinea pig. The guinea pigs were awake and little variation was found during measuring process. The result suggested the IOPs of guinea pigs in one day were normally distributed. The mean IOP was 10.3±0.36 mm Hg. In this respect, the mean IOP of adult guinea pigs by Coster *et al*[17] was 18.27±4.55 mm Hg. The tonometry we used was based on the rebound principle, while the research by Coster *et al*[17] used the applanation tonometer. It indicated the respective methods which were chosen would lead to a different result. The IOP by Goldmann applanation tonometer was reported 5 mm Hg higher than RBT in the same mice[18-20]. Therefore, our results was similar to the previous result despite different animals were selected. Moreover, our research observed the fluctuation range of IOP in guinea pigs was lower than that in humans. We speculated that it was related to the corneal thickness (CT). Research in humans by Rao *et al*[21] claimed the central CT in humans was 500 μm, while the CT in guinea pigs was 227.85±14.09 μm[22]. More work need to

do to compare the difference between Goldmann applanation tonometer and RBT in guinea pigs.

The medical treatment of beta-adrenergic blocking agents mainly came from the suppression of aqueous production, Carteolol was the most widely used[23]. In our current research, no significant difference was observed before and after treatment in Carteolol group. It suggested beta-adrenergic blocking agents had a limited effect to reduce IOP in guinea pigs.

Brimonidine was an alpha-adrenergic agonist that primarily inhibited aqueous production and increased aqueous outflow further. In Brimonidine group, the IOP was significantly lower than baseline IOP after one hour, and the significant effect lasted for two hours, which was similar to the research of Akaishi *et al*[24].

The eyeball of guinea pigs was found sensitive to Brinzolamide. Our current research observed the IOP in Brinzolamide group was decreased one hour after treatment, and lasted the longest compared to the other groups. In this respect, inhibition of carbonic anhydrase in the ciliary processes of the eye decreased aqueous humor secretion and lowered the IOP in the anterior chamber, presumably by reducing the rate of formation of bicarbonate ions with subsequent reduction in sodium and fluid transport[25]. Our research was similar to the results of Toris *et al*[26], they reported a much decreased IOP in rabbit by Brinzolamide. However, Gray *et al*[27] reported no reducing IOP effects were found after 1% Brinzolamide every 12h in normal cats. Such result indicated reducing IOP effects varied with species. In addition, we observed a rising IOP compared to baseline IOP in spite of the drug effects began to disappear. The IOP was 1.3±0.89 mm Hg higher than baseline IOP at the end of the experiment. In this point, one reasonable explanation was the Brinzolamide could decrease aqueous humor secretion and thus lower the IOP in the anterior chamber, the ciliary body might increase the aqueous humor secretion through compensatory mechanism accompanied by the attenuated drug effect. Therefore, the IOP could increase in a short time, but the detailed mechanism still needs to be further studied.

Latanoprost is one kind of prostaglandin analogs which could increase uveoscleral outflow of aqueous. It was reported that Latanaprost drops could strikingly decrease the IOP of human[28] and monkey[29]. However, no obvious effect was found in rabbit[30] and cat[31]. In the current study, Latanaprost drops could decrease the IOP of guinea pig significantly. In the study by McDonald *et al*[32] in cats, no significant difference in IOP was found after 3wk of Latanoprost drops, but the outflow of aqueous was observed increased. Such results suggested a similar mechanism between human and small animals like guinea pig.

The shortcoming of this research was, only RBT was used, and the relationship between CT and IOP was still unclear. Thus, our further work will focus on the difference between Goldmann applanation tonometer and RBT in guinea pigs, and the CT of guinea pigs by using optical coherence tomography. The individual difference of each animal also required more attention.

In summary, our work represented an attempt to expand our knowledge of baseline IOPs of guinea pigs. We found a different effect on the IOP by using four reducing IOP agents. Studies of this sort might reveal some useful features of animal eyeball, and it would also make the guinea pig a promising animal model in future studies in glaucoma.

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