A pilot study on the novel effect of Neu-P11 on reducing the intraocular pressure in normotensive rabbits

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Melatonin is involved in the regulation of intraocular pressure. To evaluate the effect of a melatonin receptor agonist (Neu-P11) on intraocular pressure, a multiple-dose study was performed in 30 ocular normotensive rabbits by regularly measuring the intraocular pressure within 7 h. The results indicated that topical applications of Neu-P11 and melatonin could both produce dose-dependent reductions in intraocular pressure with maximal reduction being observed at 1 h for Neu-P11 and melatonin in 10^{-5} mol/L. Moreover, there were significant differences in the declinations of intraocular pressure between Neu-P11 and melatonin at the doses of over 10^{-9} mol/L (p < 0.05) in the treatment group. This study suggests that Neu-P11 is a potential substance for the reducing and treatment of ocular hypertension.

Key words: Intraocular pressure, melatonin, Neu-P11, rabbit.

INTRODUCTION

Variations in intraocular pressure (IOP) are the diurnal rhythmic activities with a low level in the light and a high level in the dark in rabbits (Liu and Dacus, 1991) and reflect a balance between the inflow and outflow of aqueous humors (Civan and Macknight, 2004). Evidence has indicated that the variations of IOP are influenced by melatonin, that can regulate osmotic exchanges between the blood and intraocular fluid (Bucolo et al., 2013; Musumeci et al., 2013). Therefore, substances such as melatonin and its receptor agonists are potential drugs for the treatment of abnormal IOPs (Bucolo et al., 2013).

Melatonin is a neurohormone expressed in the tissues, including pineal gland, retina (Wiechmann 1986; Tosini 2000; Zawilska et al., 2009), ciliary body (Martin et al., 1992), and lens (Abe et al., 1999; Itoh et al., 2007). This neurohormone is involved in the regulations on rhythmic activities such as circadian seasonal rhythms (Alarma-Estrany and Pintor 2007), sleep and cyto-/neuroprotection (Hardeland 2010). So far, clinical studies have shown that melatonin could protect ocular tissues by effectively scavenging free radicals and excessive amounts of nitric oxide (NO) generated in the glaucomatous eyes (Rosenstein et al., 2010; Agorastos and Huber 2011). As the analogues of melatonin, in recent years, much evidence has indicated that melatonin agonists can activate the melatonin receptors and have therapeutic applications in the treatment on sleep disorders (Hardeland et al., 2008) and depression (Guardiola-Lemaitre, 2007; Spadoni et al., 2011). Based on the mechanics of melatonin and melatonin agonists,
we predicted that some melatonin agonists might be more available for regulating the IOPs. Melatonin agonists which might be more available for regulating the IOPs.

Neu-P11 is one of the novel melatonin agonists with high affinity to melatonin receptors. A recent study has demonstrated that the agonist can improve the energy metabolism and insulin sensitivity of eyes (She et al., 2009). Moreover, the results from animal studies have revealed that this substance has obvious effects on the treatments of antidepressant and anxiolytic activities in rodent models (Tian et al., 2010). However, its effects on IOP remained unclear although this melatonin agonist could improve the sleep of mice (Oertel et al., 2013) and energy metabolism of animals by increasing superoxide dismutase, catalase and glutathione-reductase activities (Chuffa et al., 2011). In the present study, to evaluate the effect of Neu-P11 on IOP, a multiple-dose study was performed by regularly measuring the IOPs within 7 h in 30 ocular normotensive rabbits.

MATERIALS AND METHODS

Animals

Thirty male New Zealand white rabbits (2.5 to 3.0 kg) were kept in cages individually, with free access to food and water. They were submitted to control in light/12 h and dark/12 h cycles. All the protocols and the statement for the use of animals in ophthalmology and vision research were described complying with the Association for Research in Vision and Ophthalmology (ARVO). This study was in accordance with the European Communities Council Directives (2010/63/EU).

Compounds

The Neu-P11 (C₁₇H₂₀N₂O₄) and melatonin (C₁₇H₂₆N₂O₂) used in the study were provided by Neurim Pharmaceuticals Ltd. (Tel-Aviv, Israel), and the Oxibuprocaine/tetracaine anesthetic was purchased from Cusi laboratories (Spain) (Figure 1).

Preparation for formulations

The stock solutions of melatonin and Neu-P11 were prepared by dissolving in dimethylsulfoxide (DMSO) in 1 mM. Then, the stock solution was diluted with isotonic saline solution (sodium chloride) to different final concentrations (10⁻⁴ to 10⁻⁵ mol/L). All different final concentration solutions contained less than 1% DMSO and were kept at room temperature.

Intraocular pressure measurement

The IOPs, including right eye (Oculus Dexter, OD) and left eye (Oculus Sinister, OS) were measured by using a Tonopen XL contact tonometer supplied with Mentor (USA). Each cornea was anesthetized by applying 10 μl anesthetic containing 4 mg oxibuprocaine and 1 mg tetracaine. Bilateral IOPs were measured as resting IOP before melatonin or Neu-P11 treatment was cornea unilaterally to the at a fixed volume of 10 μl, while the contralateral eye was received 10 μl physiological saline.

Pharmacological protocols

10 μl melatonin or Neu-P11 was assayed in a wide range of doses from 10⁻⁵ to 10⁻⁴ mol/L, to generate the dose-effect curves. In the experiments, IOPs were measured during the maximal effect of the agent. Moreover, to study the time-course effect of single dose (10⁻⁵ mol/L) of melatonin or Neu-P11. IOPs were regularly measured crossing 7 h. In the experiments, one single dose was tested for a single animal, and there was a 2 days interval between the dose tests for the same animal.

Statistical analysis

Statistical differences between treatments were calculated with two-way repeated-measures analysis of variance (ANOVA) by using SPSS15.0. Significant statistical difference was refered to as p < 0.05 in the study. Plotting and fitting were carried out with MICROCAL ORIGIN 7.0 (Micro Software, Inc., Northampton, MA, USA). Data was displayed as means ± standard deviations (M ± SD).

RESULTS

Resting intraocular pressure measurement

The resting IOPs of 30 male New Zealand white rabbits were measured as value at 20.65 ± 3.80 mmHg before melatonin and Neu-P11 treatments were performed.

Treatments of melatonin and Neu-P11 on intraocular pressures

Fifteen white rabbits were treated with melatonin and the other 15 were treated with Neu-P11. The fixed volumes (10 μl) of melatonin and Neu-P11 were assayed with across concentrations ranging from 10⁻¹⁴ to 10⁻⁵ mol/L. The results indicated that the two substances could both induce obvious reductions in the levels of IOPs with the increasing doses. Furthermore, the Neu-P11 treatment group, in comparison with the melatonin group, showed a significant decrease in IOP when the concentration exceeded 10⁻⁹ mol/L (p < 0.05) (Figure 2).

Time-course effect of Neu-P11 on intraocular pressures

We selected an optical dose of melatonin or Neu-P11 at 10 μl (10⁻⁵ mol/L) for unilateral eye. In the treatment, 10 μl working solution of Neu-P11 (10⁻⁵ mol/L) solution in saline DMSO was instilled in saccus conjunctivae of the contralateral eye. IOPs were measured at 0, 0.5, 1, 2, 3,
Figure 1. Formula of Neu-P11 and Melatonin

Figure 2. Dose-response curves for Neu-P11 and melatonin on intraocular pressure in New Zealand white rabbits. 10 µl of Neu-P11 and melatonin were tested at concentration range varied from $10^{-14}$ to $10^{-5}$ mol/L. Values represent mean ± SD of ten independent experiments.

4, 5, 6 and 7 h after the instillation. There was no a significant difference in the variation of IOP between melatonin and Neu-P11 in the vehicle group ($p > 0.05$). Moreover, both melatonin and Neu-P11 treatments reduced IOP rapidly at 0.5 h and maximally at 1 h with 15.46 ± 2.28 and 11.99 ± 2.45 mmHg. On the contrast, the effect of Neu-P11 was more sustained than that of melatonin. The reductions in IOPs were lasting for 3 h in melatonin group and 5 h in Neu-P11 group (Figure 3). Then, the pressures returned back to baseline.

DISCUSSION

The role of melatonin in IOP and the relationship of the variations in IOPs and the presence of melatonin receptors have been reported in previous studies (Rohde et al., 1985; Samples et al., 1988; Pintor et al., 2003; Alarma-Estrany et al., 2008; Musumeci et al., 2013). However, there was no evidence about the regulation of Neu-P11 on IOP. We found that Neu-P11 could reduce IOP in a dose-dependent manner over 5 h and showed a
Figure 3. Time-course effect of Neu-P11 and melatonin on rabbit intraocular pressure. Values represent the mean ± SD of ten independent experiments.

maximum reduction with single dose at 1 h.

Some previous studies indicated that the topical applications of N-butanoyl-2-(2-methoxy-6H-isindo1o [2,1-a]indol-11-yl) ethanamine (IIK7) and 5-Methoxycarbonylamino-N-acetyltryptamine 5-MCA-NAT) could produce lower reductions in IOP in New Zealand rabbit normotensive eyes (Pintor et al., 2001; Pintor et al., 2003; Alarma-Estrany et al., 2008). The effects were similar to the Neu-P11 in our study. These findings suggested that Neu-P11 had a hypotensive effect similar to the well-known MT-melatonin receptor agonists. So far, studies have evidenced that melatonin and its analogues can cause concentration- dependent reductions of SNP-released NO and cGMP production via activation of MT2 receptors in human NPCE cells, which play a role in melatonin agonist- induced regulation of aqueous humor secretion and IOP (Dortch-Carnes and Tosini, 2013). Therefore, we believed that Neu-P11 has similar mechanism in the regulation of IOPs as MT-melatonin receptor agonists.

In the present study, we observed that the Neu-P11 had a better hypotensive effect than melatonin. These findings provided further supports on the previous findings that melatonin analogues had an advantage over melatonin in reducing IOP (Pintor et al., 2003; Alarma-Estrany et al., 2008; Musumeci et al., 2013). In the present study, Neu-P11 produced a lasting reduction in IOP in the cornea as compared to melatonin. The result implies that Neu-P11 possibly produced a longer inhibition in the adenylyl cyclase, an increasing formation of cyclic adenosine monophosphate (cAMP) and a larger protein kinase A (PKA) activity (Dubocovich et al., 2010). Thus, we deduced that Neu-P11 was a better hypotensive substance than melatonin in the treatment of ocular hypertension. Furthermore, considering the roles of Neu-P11 in energy metabolism and insulin sensitivity, depression and anxiety (She et al., 2009), this substance would have some advantages in treatment on ocular hypertension cases with abnormal energy metabolism, insulin sensitivity and mental condition. However, further evaluator studies are required to assess the veracity of this claim.

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