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Original Research Article

Protective roles of ketamine and xylazine against light-induced retinal degeneration in rats

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Abstract

Purpose: *To study the protective effects of ketamine and xylazine against light exposure-induced retinal degeneration (RD) in rats.*
Methods: *Sprague Dawley rats were divided into three groups viz: light damage before anesthesia (LAE), light damage only (LDO), and control (CON) group which was kept in the dark for 12 - 18 h to habituate before light exposure. LDO group was exposed to light before anesthesia, while LAE group was maintained under anesthesia with ketamine and xylazine. The groups were kept for 120 min in darkness after anesthesia prior to light exposure and they were awakened prior to light damage. Functional assessment was carried out using electroretinography while morphological analysis was carried out using histology and immunochemistry techniques.*
Results: *Ketamine-xylazine combination preserved the function of the retina and protected against light-induced RD based on retinal imaging studies and immunochemistry analysis. Xylazine and ketamine anesthesia provided protection against light-induced retinal damage, and thus reduced photoreceptor cell death.*
Conclusion: *These results indicate that xylazine and ketamine anesthesia offer protection against light-induced damage and photoreceptor cell death in rats, and therefore, can potentially be developed for use in humans.*

Keywords: *Retinal degeneration, Light damage, Protection, Xylazine, Ketamine*

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INTRODUCTION

Retinal degeneration (RD) is a condition of retinopathy which leads to retinal degeneration due to death of the retinal cells as a result of loss of light-sensitive photoreceptors [1]. Photoreceptor cells are involved in the visual process. Thus, their death results ultimately in RD. In the current investigation, retinal light damage in rats was used as a model for studies on the prevention of retinal degeneration in humans using neuroprotective approaches [2]. Neuroprotection involves the preservation and protection of the structure and function of neuronal cells after an acute insult through a mechanism involving minimization of damage to these cells, and maximization of their recovery [3].

Sometimes, neuroprotection is a prophylactic

measure at the peri-ischemic period in order to enhance the survival of neuronal cells. Indeed, substantial research attention has been focused on identifying agents/chemicals agents that reduce neuronal injury during anesthesia. There are also studies on the effect of anesthetics on the physiopathology of ischemia in neuronal damage in animal models [4]. The main goal of neuroprotective treatments is to reduce cerebral damage, thereby increasing the tolerance of neurological tissue to ischemia and changing the intracellular responses to energy supply deprivation. Moreover, there are reports on the neuroprotective effect of anesthetics against ischemic insults and other neurodegenerative diseases including Parkinson disease (PD) and Alzheimer disease (AD) in rats.

The neuroprotective effects of chemical agents such as chloral hydrate-carprofen, ketamine-xylazine and fentanyl-medetomidine for anesthesia has been reported in *in vivo* studies in the retinal cells of various animal models [5]. Light has the ability to damage the retina (phototoxicity), thereby decreasing the responses of the photoreceptor to light. Among the different components of visible light, the blue colour between the wavelengths of 400 and 500 nm is considered the most harmful component. Several experiments have demonstrated the use of animal models to investigate RD caused by light damage which may occur due to external factors or diseases [6].

Studies have also shown that light-induced RD model in gas inhaled anesthesia leads to neuroprotection against photoreceptor apoptosis. The present study was aimed at investigating the protective effect of ketamine and xylazine against light-induced retinal degeneration in rats. The induction of RD was based on the protocol developed by Arango-Gonzalez *et al* [7]. The neuro-protective use of ketamine-xylazine on RD has been reported in various researches.

The present investigation focused on its potential protective effect against RD.

EXPERIMENTAL

Ethical statement

The study protocol was approved by Jilin University Ethical Committee (approval no. JUEC/ANE/12252017). All procedures were performed in accordance with the guidelines of National Institute of Health for Care and Use of Laboratory Animals [8].

Experimental animals

The animals were handled as per the National Institute of Health, USA Guidelines for Care and Use of Laboratory Animals [8]. Thirty Sprague-Dawley rats, weighing 290 – 350 g were used in the study. The rats were housed in polypropylene cages with standard laboratory conditions at an optimum room temperature of 22 ^{o}C, with a 12 h light/12 h dark cycle and 200 lux of illumination.

Anesthesia and light exposure

The rats were divided into three groups viz. LDO, LAE, and CON (Table 1). They were kept in the dark for 12 -18 h to habituate before the light exposure. Initially, the LDO group was exposed to light before anesthesia, while the LAE group was maintained under anesthesia by injecting with ketamine (80 mg/kg0 and xylazine (10 mg/kg) for at least 90 min. These groups were kept for another 120 min in darkness after anesthesia prior to light exposure. The rats were awakened prior to light-induced damage, and their pupils were dilated 40 min with tropicamide (one drop to each pupil) to allow for light damage. To prevent closure of the eyes, the rats were monitored every 15 - 20 min. Then, the LDO and LAE groups were exposed for 120 min to light with a brightness of 14,000 lux, after which they were transferred to darkness for about 14 h as per the standard protocol [9]. The rats in the control group were not exposed to anesthesia and light illumination.

Electroretinography (ERG)

Before the exposure to light, the baseline electroretinography (ERG) of the LDO and LAE rat groups were recorded every 10 days. In addition, the baseline ERG recording was carried out once every 10 days after the light-induced damage. The rats were adapted to 12 h of darkness and one drop of tropicamide was applied to the pupils. After 30 min, the measurement of ERG was carried out using an active electrode. To avoid keratopathy, methyl-cellulose was added. The electrode was introduced between the eyes and the tail to act as the reference. The ERG recording protocol comprised various steps for measuring the strength of the stimuli produced by white light using ColorDomeH Simulator. The flash duration was set at 5 ms and the scotopic flashes were delivered without any background illumination. ERG data analysis was carried out using Delphi 7.0. The oscillatory data were extracted using discrete Fourier transform (80 –290 Hz), and the a-wave and b-waves were analyzed. However, for light flashes with low intensity, the rod

sensitivity was estimated by fitting the Naka-Rushton fit [10]. Furthermore, the a-wave and b-wave were compared with those of the control groups.

In vivo retinal imaging

The LDO and LAE groups were exposed to light, and retinal imaging was conducted on the 2nd day and on the 10th day. The control groups were kept for reference. Based on the protocol described by Zhang *et al*, confocal scanning laser ophthalmoscopy (cSLO) and spectral domain optical coherence tomography (SDOCT) imaging were conducted [11]. The imaging of the central region of peripapillary was done with the near-infrared channel, while the horizontal and vertical SD-OCT sections were recorded perpendicularly. Eye Explorer 1.6 was employed for quantifying the retinal thickness based on the vertical scan. The total retinal thickness (TRT) and outer nuclear layer thickness (ONL) of the LAE, LDO, and control groups were quantified on the 8th loci towards the periphery. Furthermore, the segmentation line detected the TRT.

Histology and immunohistochemical analysis

The rats were sacrificed without any physical pain and the lenses and the anterior parts were excised from the eyes. They were further treated with 4 % PFA in 0.1 M PO_4 at 4˚C for 60 min and dehydrated in ethanol. They were then embedded in paraffin after immersing in chloroform. Paraffin sections were radically sliced at 5 mm and kept at 4˚C. The ONL, thickness and the segments were quantified and at equal distances. For immunohistochemical studies, the paraffin sections were washed and rehydrated. The antigen was retrieved by treating with 0.1 M citrate buffer, pH 5.8 under pressure for 12 min. This was followed by cooling the section with the specific antibodies. The radial sections were further treated with PBS (70 mM, pH 7.2) consisting of 0.05 % Triton X-100 and goat serum (15 %) for 120 min. The sections were then incubated at 4 ^{o}C with their corresponding antibodies for 24 h. The reactions were observed with a standard anti-rabbit antibody (Alexa Fluor 488) diluted in the ratio of 1: 600. The sections from the controls were processed without anti-rabbit antibody. Photomicrographs were recorded for the superior region of the retina using a microscope.

In each group, data were obtained from three different rats chosen randomly. The data analysis also includes measurement of the control rats for evocative assessment. For the quantification of the captured cells, snapshots of whole radial slices were magnified and the cells were manually counted. The total cells were calculated by the dividing the ONL with average cell size, and with total ONAL to obtain the percentage value

Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS Inc, USA). The ERG parameters were analysed based on the ratio between baseline data and follow-up period. Data analysis for histochemical studies was carried out using *t*-test.

RESULTS

There was no statistically significant difference in the ERG response between the retinas of the LDO and LAE groups in the baseline observations and measurements. Figure 1 shows the ERG curves of the LAE and LDO groups based on the function of scotopic sensitivity. The results show a positive influence on the function of rod on-bipolar cells and on Muller cells. The analysis of variance also revealed that the rod cells of the retina were reduced in quantity, but their function was not affected. Figure 2 depicts the comparison of the a-wave amplitudes of LDO and LAE groups based on the strength of the stimulation. It is observed in Figure 2 that the a-waves showed higher amplitudes in the case of LAE group, when compared to the LDO group, due probably to scotopic conditions. These might be due the functions of retinal rods and cones which are photoreceptors for low light levels. There was not much difference in the a-wave implicit times in the LAE group, an indication of its photoreceptor protection capability. However, the gradient lines of a-wave were high for the LAE group on the 13th day after the exposure to light. The a-wave is an indicator which is electrophysiological in nature and it represents the process of photo-transduction in the sensory neurons of the retina. Thus, photo-transduction and the photoreceptor cells of the retina were preserved in this group.

Table 1: Rat groups used in the study

Group	Light exposure	Ketamine-xylazine treatment
Light damage before anesthesia (LAE)	Positive	Positive
Only light damage (LDO)	Positive	Negative
Control (CON)	Negative	Negative

Figure 3A shows that the amplitude of b-wave ratio for the LAE group had a high-intensity stimulus, when compared to the LDO group, as evidenced from Figure 3B.

The *in vivo* retinal analysis on TRT and ONL during the 2nd and 10th days is depicted in Figure 4 (A and C). The graph shows that there was no significant difference in the retinal layering, when compared to the control rats. On the other hand, rats in the LDO group showed a significant difference in ONL thickness, when compared to rats in the LAE group. However, TRT thickness was comparable between the two groups. Ten days after the light damage (Figure 4B and D),

TRT and ONL thickness were reduced in the LDO group, when compared to the LAE group. Thus, the *in vivo* retinal analysis suggests that the formation of retinal edema on the 2nd day after the light-induced damage, as well as the reduction in the ONL thickness may cause the death of photoreceptor cells.

Figure 5 depicts retinal section of the light-induced damage after 36 h. There was no significant difference in GFAP staining between the LAE and LDO groups, as depicted Figure 6. Moreover, rhodopsin stain revealed that there was not much difference in immune-labelling between the two groups (Figure 7). The rhodopsin stain also depicted a normal distribution which was restricted to the photoreceptor OS.

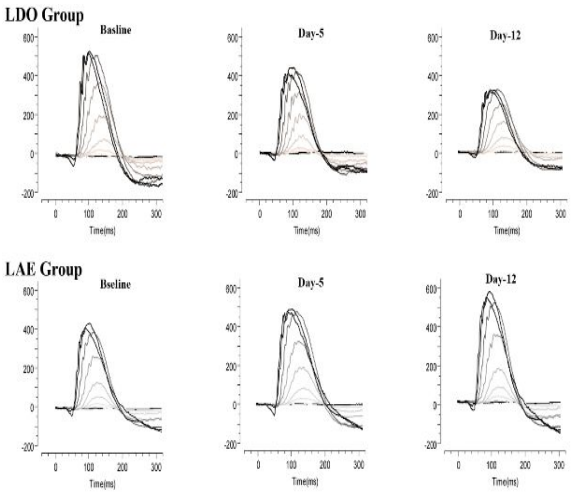

Figure 1: Responses of ERG curves to nine flashes recorded at baseline, day 5 and day 12

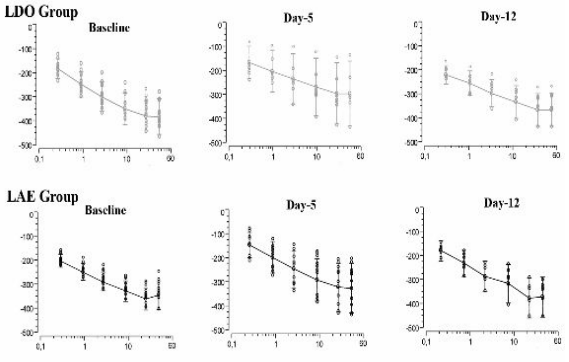

Figure 2: Comparison of amplitudes of a-wave between LDO and LAE groups

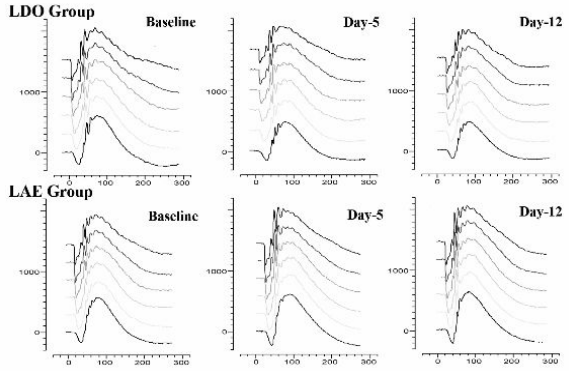

Figure 3: Comparison of amplitudes of b-wave between LDO and LAE groups

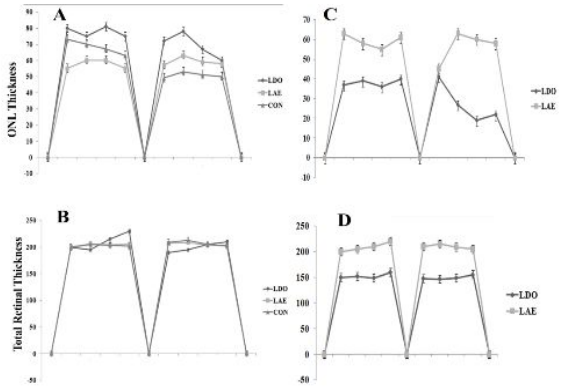

Figure 4: *In vivo* analysis of LDO, LAE and control groups based on total retinal thickness

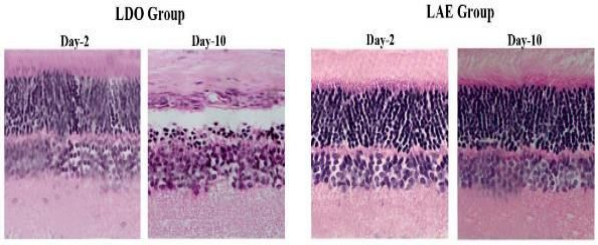

Figure 5: Hematoxylin and eosin (H&E) staining of retinal sections from LDO and LAE groups

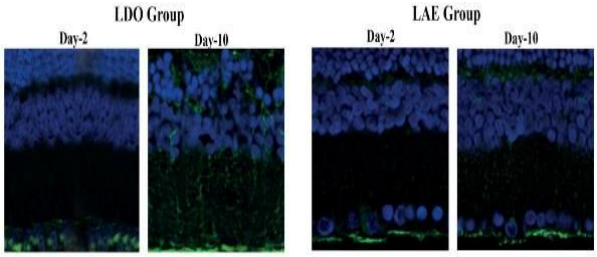

Figure 6: GFAP staining of retinal sections from LDO and LAE groups

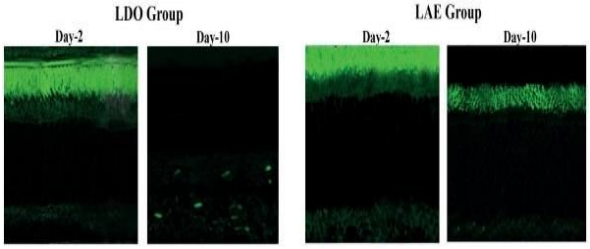

Figure 7: Rhodopsin staining of retinal sections from LDO and LAE groups

DISCUSSION

This study investigated the protective effect of ketamine and xylazine on light-induced retinal damage. Previous studies have been carried out on potential protective effects of anesthesia against retinal degeneration. Some studies have proposed mechanisms involved in the positive outcome of ketamine-xylazine anesthesia [12]. There are also reports which indicate the role of anesthetic agents as neuroprotective drugs [13]. On the other hand, various workers have reported contrasting results from studies on the effect of anesthetics on light-induced RD, thereby making complicated the understanding of the various findings [14]. Moreover, these investigations used different procedures and parameters to determine RD [15]. In addition, the tools and techniques used in various anesthetic procedures for light damage induction are different [16].

However, all the studies reported on the neuroprotective effect against light-induced RD in various animal models. Keller *et al* reported that halothane anesthesia reversibly inhibited metabolic regeneration of rhodopsin, thereby preventing further absorption of photons during the exposure of light [17]. In their study, Keller *et al*. found that halothane anesthesia prevented retinal degeneration induced by white light, but not that induced by blue light. Their study further observed that the halothane-anesthetized photoreceptors of mice and rats were protected from white light-induced RD but not from blue-light-induced damage to retinal cells [17]. This is because halothane cannot protect the retina from blue light which has the capability of restoring the function of rhodopsin from photo-reversal bleaching. This process usually allows rhodopsin molecules to capture light molecules required for the induction of RD [18].

Rhodopsin is a light-sensitive receptor protein which main function is transduction of visual signals. It is usually present in the rods of the retina which are very photo-sensitive, and constitute the major factor involved in visibility in low-intensity light environments [19]. Mutation of rhodopsin gene results in various retinopathies. In many cases, the protein arising from the mutation accumulates with ubiquitin thereby damaging the whole network cycle. In some cases, the cells are unable to degrade this mutated non-functional protein, resulting in apoptosis of the photoreceptor cells [20].

In addition, Grimm *et al* observed that exposure of rats to blue light rats caused severe retinal damage and activation AP-1 transcription factor [21]. It was also observed that green light exerted no severe effect [21]. However, in the present study, exposure of light quickly resulted in RD which can be compared to damage after mild exposure to blue light. It was also observed that the dosage of ketamine-xylazine used was sufficient to prevent the light damage. A similar effect was also observed by Keller *et al* who used halothane anesthesia [17]. This may be because ketamine alone has several limitations and it is known for its weak muscle relaxant properties. It is mainly used with a mixture of other anesthetics e.g. diazepam or xylazine [22]. On the other hand, xylazine is an agonist of the α2 class of adrenergic receptors. It produces sedation and muscle relaxation in various non-human mammals such as horse, cattle and cats. In the absence of xylazine, the neuroprotective effects of ketamine are still observed in some experiments, which suggests that xylazine has nothing to do with the neuroprotective effect. Thus, ketamine plays important role in the neuroprotection of RD [23].

The main objective of the present study was to determine the positive outcome of the use of ketamine-xylazine as anesthesia, and its role in neuroprotection in a light-induced RD model. An obvious neuroprotective effect of ketamine-xylazine was observed. These findings also provide insights into the existing reports on the implications of light damage for inducing RD in various animal models.

On the other hand, there are also reports which show that rhodopsin regeneration in rats is

slowed down by ketamine-xylazine combination [15]. It has been reported that ketamine-xylazine combination protects against light-induced damage caused by the activation of NOS. Moreover, ketamine preserves the morphology of the neuronal cells by increasing their viability [24]. This is because ketamine downregulates the expression of proteins that control the transcription of DNA such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). In many cases of neurodegenerative orders (including retinal degeneration), NF-κB is activated [25].

The present study observed a specific position in both the LAE and LDO rats in their retina. These regions may be responsible for the light-induced damage and thinning of the retinal cells. The molecular process involved in this particular damage in this localized region is not clearly understood. It may be caused by the higher level of rhodopsin.

Study limitations

The population size of the rats used in this investigation is too small for statistical and morphological analyses. Moreover, the biological relevance of neuroprotection requires further studies to unravel the biochemical mechanisms involved.

CONCLUSION

The study demonstrates that xylazine and ketamine anesthesia provide protection against light-induced retinal damage, and reduces the death of photoreceptors in rats. Thus, the use of the anesthetic mixture has clinical potentials for its neuroprotective effect in humans.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work. The funding organization(s) played no role in the study design nor in the collection, analysis, and interpretation of data; writing of the report or in the decision to submit the report for publication.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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