• Basic Research •

Acute lens opacity induced by different kinds of anesthetic drugs in mice

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Abstract

• AIM: To study whether specific anesthetic drugs or tear layer evaporation was primarily responsible for the acute cataract and what the change of lens structure is in anesthetized mice.

• METHODS: Five groups were set up in the experiment: Group A (topicamide and phenylephrine mixed eye drop+ chloral hydrate), Group B (tropicamide and phenylephrine mixed eye drop+sevoflurane), Group C (tropicamide and phenylephrine mixed eye drop), Group D (topicamide and phenylephrine mixed eye drop+chloral hydrate, carbomer eye drop in the right eyes), and Group E (tropicamide and phenylephrine mixed eye drop+sevoflurane, carbomer eye drop in the right eyes). A simple classification system was used to assess the severity of lens opacity. And a numerical value from 0 to 3 to each grade was assigned for the cataract index calculation and data analysis. The gross appearance and time course of development of lens opacity were assessed. Hematoxylin and eosin staining was used to observe the lens structure changes in the reversible cataract.

• RESULTS: Tropicamide did not induce lens opacification in mice. Lens opacity caused by inhaled sevoflurane was similar to injected cholral hydrate. Both inhaled-anesthetic-induced lens opacity and injected-anesthetic-induced lens opacity could be prevented by carbomer eye drop. In the severe opacity lens, a wide range of lens fiber cell structure had disordered. The fiber cells became uneven thickness.

• CONCLUSION: The acute reversible lens opacity can unilaterally develop or be induced by a local cause. The structure of lens fiber cells changed in the lens opacity which may influence the permanent connection of the lens fiber cells. This study was not only of practical significance to help maintain lens transparency for eye research, but also of the deeper consideration about the reversible lens opacification phenomenon.

• KEYWORDS: lens; opacity; anesthetic drugs; tear film; mice

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INTRODUCTION

Mouse is the animal models commonly used in ophthalmology research[1-3], and general anesthesia is often required *in vivo* experiment[4-5], such as fund us examination or visual stimulate. Therefore, the feasibility of iatrogenic lens opacity must be considered which may have a significant effect on the result of measurement and even the experimental accuracy. It has been reported that several drugs could induce the acute lens opacity in animal models of ophthalmic research such as phenylephrine, sodium selenite, naphthoquinone, xylazine and ketamine, etorphine, phenelzine and serotonin, adrenaline, and chloral hydrate[6-9]. Early studies have shown that a range of exogenous factors can affect the transparency of the lens, such as drugs, anesthetics, temperature, and so on[10-14]. Fluid homeostasis, especially water cycle and ion exchange also have a critical effect on lens transparency[15-18]. As a result, it is still challenging to clarify the exact causes of reversible lens opacification in the anesthetized mice, the anesthetics or fluid homeostasis changes or both.

In this study, we were to test whether specific anesthetic drugs or tear layer evaporation was primarily responsible for the development of cataracts in mice. More importantly, we showed what kind of changes took place in the lens structure. This study was significant to help maintain lens transparency in the ophthalmic research.

MATERIALS AND METHODS

Ethical Approval All of the procedures involving animals

met the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Vision and Ophthalmic Research which were approved by the Animal Use Committee of the Institute of Zoology, Chinese Academy of Science.

Animals Four-week-old male C57BL/6 mice were obtained from Department of Laboratory Animal Science; China Medical University and brought up in the specific pathogen-free circumstance which was maintained at 21℃±1℃ temperature and 40%-70% humidity with a 12-hour light-dark cycle. All mice were accessed freely to standard chow and drinking water in ventilated polycarbonate mouse cages.

Experimental Design Thirty-five mice were used in the experiment (aged four weeks, weight 25±3.5 g). The research was at a comparatively unchanging 23℃ temperature and 55% humidity. They were divided into five groups (Groups A to E). Five minutes before anesthesia, the pupils were dilated with 0.5% tropicamide/0.5% phenylephrine mixed eye drops in all groups. Groups A, B and C were to research the process induced by chloral hydrate and sevoflurane. If maintaining the stability of tear layer can prevent cataract formation was explored by Groups D and E. The mice of Groups A and D were injected by 4% chloral hydrate solution to anesthesia. The dose was 10 mL/kg[8,19]. The mice in Groups B and E exposured to 2 L/min air with 2.5% sevoflurane for one hour. Other groups (Groups A, C and D) of mice inhaled the air without 2.5% sevoflurane[20-21]. The right eyes in Groups D and E were dripped with carbomer eye drop (Vidisic, Bausch+Lomb, Germany) respectively every 30min. Each mouse was used only once in the experiment.

Assessment of Lens Opacity The eyes were inspected by the slit lamp at 0, 15, 30, 45, 60min after inhaled 2 L/min air and 0, 30, 60, 120, 180, 240min from recovering after anesthesia. A simple classification system was used in our experiment to assess the severity of lens opacity which was previously described by Bermudez *et al*[13]. Figure 1 showed the grades of lens opacity. The digit from 0 to 3 was used to quantify the cataract development for the data analysis.

Hematoxylin and Eosin Staining The eyeballs of mice in Group E were got after cervical dislocation. They regulared in 4% paraformaldehyde at 4℃ temperature overnight. Specimens then underwent graded alcohols dehydration, clearing in xylene and embedded in paraffin. The 4-μm sections were cut for using. Sections were stained with hematoxylin and eosin and pictures were taken using an Olympus light microscope equipped with a Spot CCD camera.

RESULTS

Characteristics of Reversible Lens Opacity A rapid mydriasis was obtained by insallation of tropicamide lasted for 6h in all mice. No opacity was noticed after mydriasis in the five groups. After receiving the anesthetics, the lens showed anterior subcapsular opacity from peripheral to central. Eventually, the entire lens became opaque and showed a diffuse milk-white opacity (Figure 1).

Development and Reversal of Opacification In Groups A and B lens opacification was observed among most mice. No mice in Group C developed visible lens opacity. In Group A, the lens opacity was noticed at 15min and progressed rapidly, with 2/7 lens developed mild opacity, and 3/7 lens showed medium opacity at 30min. All mice developed lens opacification at 60min. The entire lens showed a milk-white suffused opacity. In Group B, the course of lens opacity was the same as that in Group A. The 3/7 lens developed mild opacity, and 2/7 lens showed medium opacity at 30min. The 6/7 lens showed severe opacification at 60min (Figure 2).

After the mice recovering from the anesthesia (Figure 2), the lens opacification started to be reversed. Both in Groups A and B, the opacity needed more time to reverse than develop. The lens opacification had not changed in 60min. In Group A, one lens completely reversed at 120min and 6/7 lens was transparent at 240min. In Group B, all lens completely reversed at 240min. The lens opacification had no apparent difference between Groups A and B (Figure 2). Both injected or inhaled anesthetic drugs could cause the acute reversible cataract.

Both in Groups D and E (Figures 3-5), the left eyes of mice natural which were exposured all developed lens opacification while in the right eyes used carbomer eye drop, no lens developed opacification. It demonstrated that tear layer evaporation might be the main cause of the reversible lens opacification in anesthetized mice.

Structure Changes of the Reversible Lens Opacity In Figure 6, a wide range of lens fiber cells distributed disorderedly in the left lens of Group E. The fiber cells became uneven thickness.

DISCUSSION

Topical phenylephrine induced lens opacity was reported[9]. Hubert *et al*[22] used 3000 mice to dilated in the research, and 19% of the mice had cataract. However, this might be known as naturally lens opacification, and did not seem to be related to the drug. In this study, tropicamide did not induce cataract in a mouse. It seems safe to use tropicamide in the ophthalmic research. Inhaled sevoflurane and injected chloral hydrate could affect the transparency of lens in most cases. The lens showed anterior subcapsular opacity from peripheral to central and progressed rapidly until the entire lens became opaque. The lens opacification started to develop about 15min after anesthesia. After the mouse recovered from the anesthesia, the cataracts gradually recovered. It has been reported that several drugs could induce the acute lens opacity in animal models of

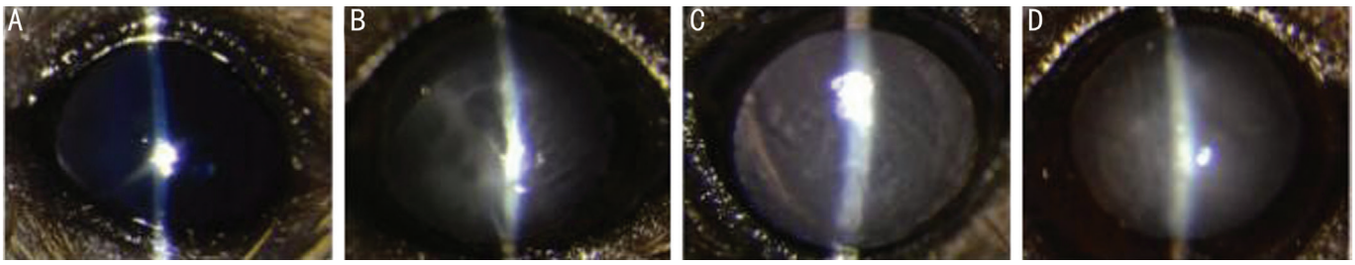

Figure 1 Four degrees opacification in lens A: No opacification: transparent lens (numerical value=0); B: Mild opacification: opacification emerged in the peripheral region (numerical value=1); C: Medium opacification: opacification emerged in the medium region (numerical value=2); D: Severe opacification: opacification emerged in the entire region (numerical value=3).

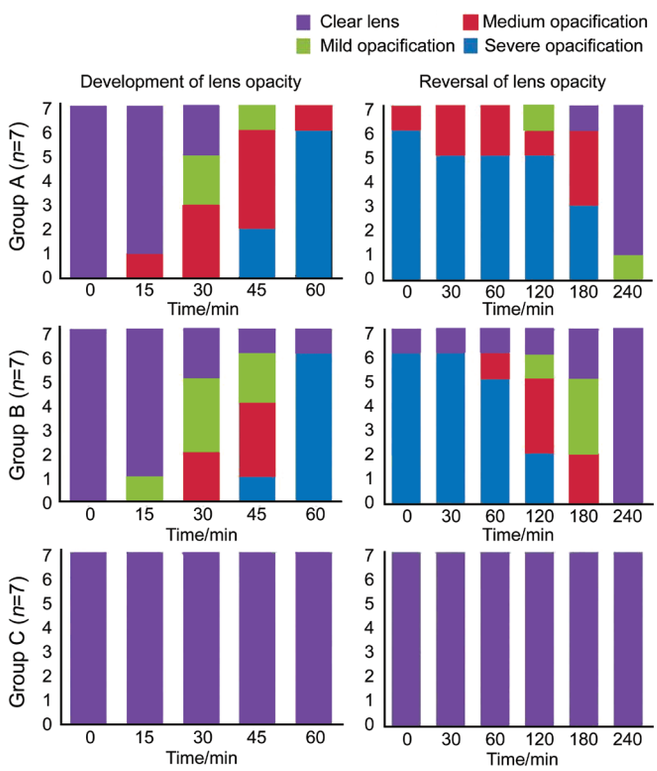

Figure 2 Time course of reversible lens opacity in the three groups (A-C) are depicted by the column charts.

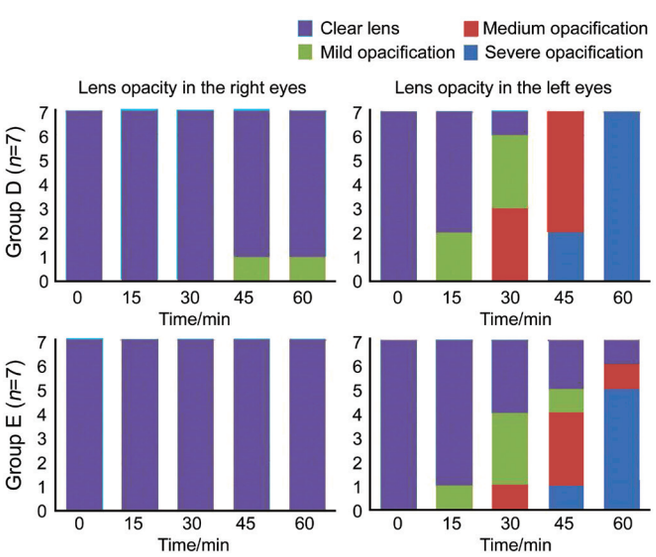

Figure 3 Time course of reversible lens opacity in Groups D and E Carbomer eye drop coverage (right eyes); natural exposure (left eyes).

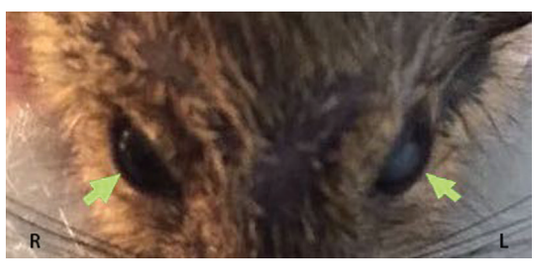

Figure 4 An example in Group E Carbomer eye drop coverage (right eyes); natural exposure (left eyes). No opacification and opacification were in the same one.

ophthalmic research including phenylephrine, sodium selenite, naphtoquinone, xylazine and ketamine, etorphine, phenelzine and serotonin, and adrenaline[6,7,10-11,14]. However the symptom caused by different types of drugs was similar[6-11,16]. Therefore, this phenomenon was more likely to be caused by the common side effects of the anesthetic drugs.

Early studies had shown that various factors affect the transparency of the lens, such as oxygen, pH, calcium, dehydration, and temperature[10-14]. Further studies were conducted on generalization and mainly focused on narcotic effects, corneal dehydrationan and temperature. Koehn *et al*[23] proposed that anesthesia can affect intraocular pressure and develop corneal lesions. In addition, fluid homeostasis[24], especially water cycle and ion flow had a critical effect on lens transparency[15,25-26]. Thus, we applied carbomer eye drop to guard against tear evaporation or corneal dehydration. Finally, it could vaildly prevent opacification. This study demonstrated that this opacification was more likely induced by local reasons than systemic factors. The anesthetic drugs could retract the eyelids, restrain the blink reflex and injure the tear film. Therefore, it is more likely that the side effects of anesthetic affect the microcirculation of the eyes, thus affecting the lens, rather than the direct effect of anesthetic on the lens. In this study, the strategy which could prevent the evaporation of tear and the dehydration of cornea could prevent opacification formation. Thus, tear film stability might be more appropriate to explain this phenomenon.

As we know, lens proteins represent 30%-35% of the total mass; the remaining 65%-70% is water compared to 95%

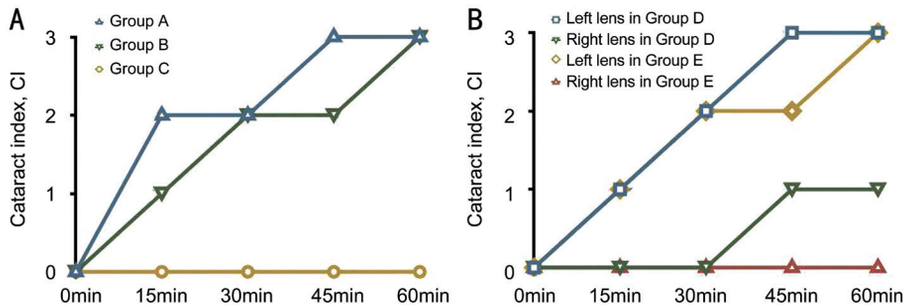

Figure 5 Time course of the reversible lens opacity in the different groups.

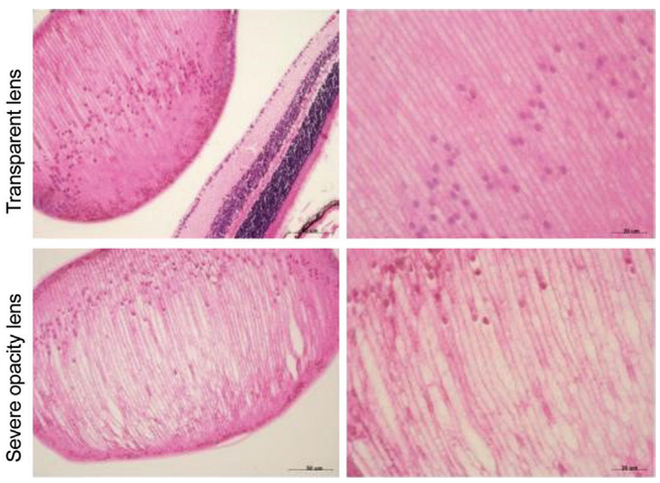

Figure 6 The lens structure in Group E The right lens were transparent, and the left were severe opacity lens.

water found in non-lenticular cells. The main constituents of the lens proteins are water soluble structural proteins, referred to as crystallins. The transparency and high refractive index of mammalian lenses are due to very high concentrations of crystallins in the lens fiber cells. However, crystallins are stabilized under appropriate conditions of osmotic pressure and PH[27]. The early study had shown that the corneal exposure could induce sodium concentration in aqueous and lens changes[28]. Variation of aqueous or osmolarity in lens influenced lens opacity[29]. These studies proved regular circulation, stable water and ion exchange in lens were significant to safeguard transparency. Besides, we observed the lens fiber cells distributed disorderedly in the cloudy lens. Also the fiber cells became uneven thickness. So the rapid reversible lens opacity can be restored in four hours. So we speculated that the changes in liquid environment interrupt the stability of lens, and then a reversible change occurred in lens fiber cells. As far as we know, this is the only research to report the lens structure changes in the reversible cataract.

In conclusion, both the inhaled anesthetics and the injected anesthetics could induce the lens opacification. However, carbomer eye drop could prevent the acute lens opacity. These supported that this opacification could independently developed by a local reason. Furthermore, we found the structural of lens fiber cells changed in the lens opacity. The structure changes of lens fiber cells might influence the permanent connection of the lens fiber cells. It was worthy considering whether these changes could affect the forward transparency of the lens. More importantly, this kind of lens opacity might be not really reversible. The research was not only of the practical significance to keep lens transparency, but also of the more in-depth consideration of the reversible lens opacification.

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