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Mobility-confinement stress produces increased light scatter in rabbit lenses

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Background. To characterize the changes in forward light scatter in the lens of the rabbit eye brought about by mobility-confinement stress.

Materials and methods. Eight Chinchilla male rabbits (weight 2.5–3.0 kg) were immobilized in narrow metal cages for 48 days. They could not move but could freely eat and drink. Eight control rabbits that had not been submitted to movement restriction were kept in normal animal room condition. Following the mobility-confinement regime (after 48 days) the rabbits (stressed and control) were anaesthetized using thiopental sodium (35 mg/kg). On removing their eyes, the lenses were prepared for light scatter studies. Forward light scatter was characterised using a 685-nm continuous-wave laser (the output power of 3 mW). The fresh – immediately after the preparation – lenses located on a special mount were put into the pathway of the laser beam. The laser beam induced a scattered pattern of light on the screen. Sony 5.1 mega pixels resolution digital camera was used to record the images.

Results. In some lenses affected by mobility-confinement stress, light scatter increased more in the regions of the cortex and the nucleus of the lens, while in the other lenses the scatter increased uniformly in all the areas, or showed scattering focuses of light in the cortex area, in comparison with that in the lenses of the control rabbit.

Conclusions. Mobility-confinement stress caused light scatter in the eye lenses of rabbits. The intensity of light scatter in the lenses affected by mobility-confinement stress changed differently in separate areas. Increased light scatter in stress-affected lenses can reflect the early stage of cataract and can be applied in practice.

Key words: mobility-confinement stress, eye lens, laser, scatter of light

INTRODUCTION

As a result of technical and social progress, people more frequently experience various stresses such as psychological or ecological ones, or those related to increasing noise, physical inactivity etc. The main factors that stimulate physical inactivity are the consequences of the industrial revolution, i.e. the elimination of hard physical work and low energy input in the working activity. New technologies (television, computer communications, electronics in workplaces, computer games, the Internet etc.) significantly decreased people's physical activeness in their professional activity and during their leisure time (1). Hypodynamics may induce metabolic disturbances and ultrastructure damage in different body systems. For instance, physical inactivity evokes arterial hypertension, hypercholesterolemia, and overweight (2), damages the ultrastructure of cardiomyocytes, microfilaments, and causes strongly expressed contractility of smooth muscles in blood vessels (3, 4). In the lens cells, immobilization stress results in vacuolization, calcification, and formation of high-molecular-weight aggregates of crystalline (5). The above-mentioned injuries within lenses may have influence on light scatter, which may lead to cataract formation (6).

The aim of the study is to characterise the changes in forward light scatter in the lens of the rabbit's eye brought about by mobility-confinement stress.

MATERIALS AND METHODS

Induction of mobility-confinement stress

The animals were cared for, used and sacrificed according to the rules defined by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (License No. 0006).

Mobility confinement stress of 48 days was provoked according to Fiodorov (7) in Chinchilla male rabbits (weight 2.5–3.0 kg; n = 8) by placing them in metal hutches, which closely shrouded their body, but the rabbits could freely feed and drink. Rabbits (n = 8) of the control group (non-stressed) were kept under normal animal room conditions. Stressed rabbits (after 48 days of the mobility-confinement regime) as well as the non-stressed (control) ones were sacrificed using thiopental sodium

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(35 mg/kg). On removing their eyes, the lenses were prepared for light scatter studies.

Investigation of the light scatter in the eye lenses

Light scattering technique is widely used for measurements of colloidal nanoparticle size and molecular weight in solution, as well as for the characterization of the protein aggregation and conformations (8, 9). As the scatter of light in the lenses may be related to changes in the protein structure, we used a 685-nm continuous-wave laser (the output power of 3 mW) to characterise forward light scatter. The image of coherent light scatter can contain useful information about damages in lens at the molecular level, associated with cataract start.

The scheme of the experimental arrangement used in our investigations is presented in Fig. 1. The fresh – immediately after the preparation – lenses (the diameter about 8 millimetres) located on a special mount were put into the pathway of the laser beam. The laser beam of the diameter 2 millimetres induced a scattered pattern of light on the screen. In order all the area of the lens was illuminated, the position of the lens was corrected by a special device. The distance between the position of the lens and the screen was selected to be 1 meter, with the aim to get a good spatial scatter resolution pattern on the screen. Sony 5.1 mega pixel resolution digital camera was used to record the images for comparative investigations.

RESULTS

The characteristics of the laser light scatter in the control lenses and lenses of the rabbits affected by mobility-confinement stress were recorded. The intensity of light scatter in the lenses affected by mobility-confinement stress changed differently. In some lenses, light scatter increased in the cortex and the nucleus regions (Fig. 2), while in other lenses the scatter increased almost uniformly in all the areas (Fig. 3). In some lenses, intensive local light scatter in the zone of the cortex could be observed (Fig. 4). Light scatter in the lenses of the control rabbits (Fig. 5) was not characterized by the changes detected in the lenses of the stressed rabbits.

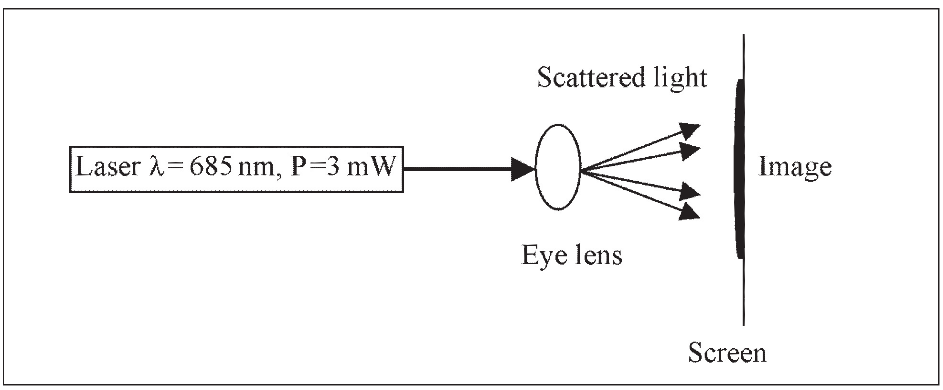

Fig. 1. Experimental arrangement with the laser to record the pattern of forward scatter produced by each sample lens

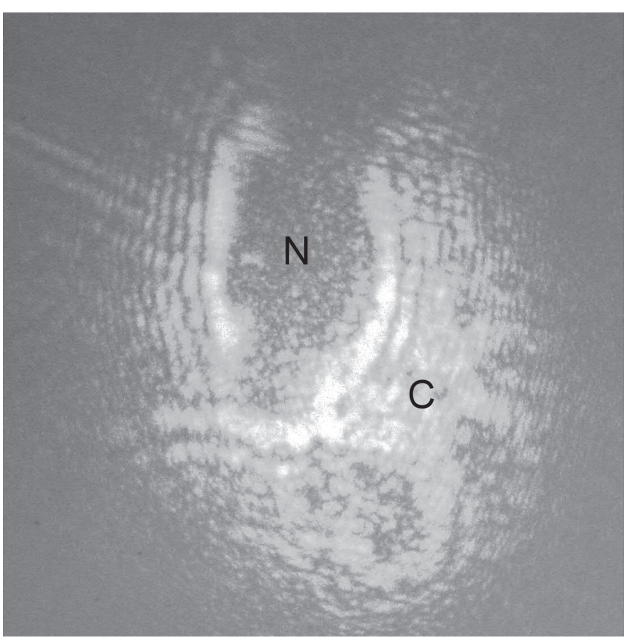

Fig. 2. Increased light scatter in the regions of the cortex and the nucleus of the lens

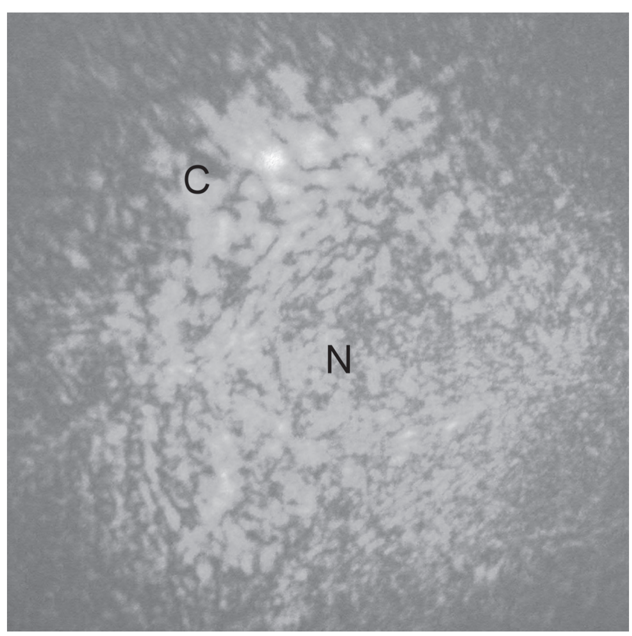

Fig. 3. The intensity of light scatter is almost uniform in all areas of lens

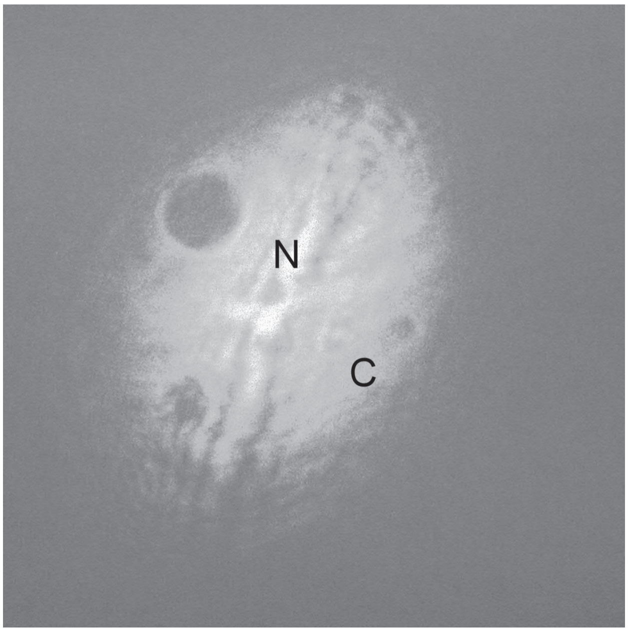

Fig. 4. Intensive local light scatter in the zone of the cortex

Fig. 5. The intensity of light emanation in the lenses of control rabbits

Note. N – nucleus, C – cortex in Figs. 2–5.

DISCUSSION

In the present study we report on the effect of a 48-day mobility-confinement stress on the light scatter in the eye lenses. As mobility-confinement stress can increase the level of catecholamines, blood pressure, and change the concentration of steroids (7), it might induce osmotic stress i. e. destabilization and disintegration of cell membranes, consequently disturbing the balance of water and elements, especially calcium. Physical stress due to immobilization (lasting for 1 hour) increased intraocular pressure in rabbits (10). A 48-day mobility-confinement stress induces vacuolization and calcification of the cortical cells of the lens (5). An increased concentration of calcium in the lenses of rabbits under a 48-day mobility-confinement stress was also determined by an atomic absorption method (11). According to Cekic (12) the calcium content was significantly higher in the lenses with cortical cataract. Electron microscopy also showed that the opacities of lens are filled with membrane-bound vesicles, containing elevated levels of free Ca^{2+} (6). Disturbances in calcium homeostasis are associated with various forms of cataract; e. g. C^{a2}+-mediated disintegrative globalization of fibres may be responsible for the formation of light scattering centres during cataractogenesis (13). In our previous experiment of 48-day mobility-confinement stress (5), changes in the structure of cortical fibres, i. e. the formation of irregular borders, compaction in an accordion-like fashion, particularly elongation with decreased interdigitations, may be related to the fibre growth (6) and aggregation of lens-specific proteins (14). Both rabbit and guinea pig lenses undergo calpain-induced proteolysis of crystalline and alpha-spectrin upon the elevation of lenticular calcium, and this may promote light scattering (15). The low-high density material, which might have been formed of proteins aggregated with calcium, was also detected in lenses under 48-day mobility-confinement stress (5). Thus, the metabolic disturbances and the ultrastructure damage in lenses under 48-day mobility-confinement stress might cause the formation of light scattering centres such as high-molecular-weight crystalline aggregates. The above-mentioned light scatter intensity changes in different areas of stress-affected lenses (Figs. 2–4) may be related to various concentrations and localizations of scattering centres. The lens of a young human is colourless and transmits almost 100% of the incident light. With age as the risk factor for cataract (16, 17), there is an increased scatter and absorption of optical radiation by the lens, and the lens becomes yellow and fluorescent. Scatter increases in all the zones of the lens. The greatest increase in scattering over time is encountered in the zone of the deep cortex, followed by the nucleus (18, 19). Increased scattering has been attributed to the accumulation of crystalline aggregates within the lens (20) and may be regarded as the precursor of cataract formation (6). Thus, increased light scatter in stress-affected lenses may reflect the early stage of cataract.

CONCLUSION

The long-term mobility-confinement stress increased light scatter in lenses of rabbits compared with that in the lenses of control rabbits. The intensity of light scatter in lenses affected by mobility-confinement stress changed differently in separate areas. So it may be related to various localizations, concentrations and nature of scattering centres: for instance, in some areas of the lens content of calcium, vacuolization may be increased, while in others, the formation of aggregates of lens proteins with calcium, changes in the structure of cortical fibres may occur.

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STRESAS DĖL APRIBOTO JUDĖJIMO SUKELIA ŠVIESOS IŠSKLAIDYMĄ TRIUŠIŲ LĘŠIUKUOSE

Santrauka

Tikslas. Charakterizuoti pirminio šviesos išsklaidymo triušių akies lęšiukuose pakitimus, kuriuos sukėlė stresas apribojus judėjimą.

Medžiaga ir metodai. Šinšilos veislės triušiai (n = 8; 2,5–3,0 kg) buvo imobilizuoti 48 paras siauruose metaliniuose narveliuose. Gyvūnų judėjimas buvo apribotas, o ėsti ir gerti galėjo laisvai. Kontroliniai triušiai taip pat buvo aštuoni ir tokio pat svorio. Jie judėjo laisvai ir buvo laikomi normaliomis vivariumo sąlygomis. Po 48 parų visi triušiai (imobilizuoti ir kontroliniai) buvo užmigdyti tiopentaliu (35 mg/kg dozė). Iš akių išimti lęšiukai (sausi specialiuose įtvaruose) buvo dedami 685 nm bangos ilgio lazerio spindulio (2 mm skersmens) kelyje. Atstumas tarp lęšiuko ir ekrano buvo vienas metras. Vaizdus fotografavome filmavimo kamera (Sony 5.1 mega pixel).

Rezultatai. Nustatėme, kad streso paveiktų triušių lęšiukuose šviesa buvo labiau išsklaidyta žievinėje ir branduolinėje dalyse, kitų – visose srityse. Kai kuriuose lęšiukuose išryškėjo intensyvus lokalus šviesos išsklaidymas žievinėje dalyje. Kontrolinių triušių lęšiukuose šviesa nebuvo išsklaidyta.

Išvados. Stresas dėl apriboto judėjimo sukelia intensyvų šviesos išsklaidymą triušių akies lęšiukuose. Šviesos išsklaidymo intensyvumas įvairiose lęšiukų zonose kinta skirtingai ir gali priklausyti nuo šviesą išsklaidančių centrų lęšyje lokalizacijos, koncentracijos ir jų prigimties: padidėjusių kalcio sankaupų, vakuolių vienose lęšio srityse, agregatų iš kalcio ir proteinų formavimosi kitose, žievės skaidulų struktūros kitimo.

Raktažodžiai: stresas dėl apriboto judėjimo, akies lęšis, lazeris, šviesos išsklaidymas