BIOPHYSICS AND BIOCHEMISTRY

Inhibition of Cataractogenesis in Congenitally Cataractous Mice (Cat fraser Strain) by an Antioxidant from the Group of Sterically Hindered Phenols

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The phenolic antioxidant catavidan, used to treat heterozygous male Cat fraser mice for 4 months starting from the age of 2 months, inhibited cataract development in these mice significantly (Z=0.063 using the nonparametric Mann-Whitney test) after 2 months of treatment. However, the differences between the treated and control (untreated) mice became less marked toward the end of the third month and disappeared by the end of the fourth.

Key Words: *phenolic antioxidant; hereditary cataract; Cat^{Fr} gene*

Mice of the strain Cat fraser carry the autosomal gene Cat^{Fr}, which determines the presence of congenital cataract. The crystalline lens of homozygous Cat fraser mice, unlike that of BALB mice, has been shown to contain elevated levels of free-radical oxidation (FRO) products such as conjugated dienes and ketodienes, and of terminal fluorescent lipofuscin-like products [4]. This led us to assume that if the accumulation of FRO products in the lens or the FRO itself play an important role in cataractogenesis, then this process might be mitigated through inhibition of FRO by an antioxidant.

To check this possibility experimentally, we chose mice heterozygous for the Cat^{Fr} gene because the rate of cataract development and the severity of lens damage in such mice are significantly less than in their homozygous counterparts. A water-soluble antioxidant from the class of sterically hindered phenols was used (synthesized by A. A. Volod'kin at the Institute of Chemical Physics, Moscow).

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MATERIALS AND METHODS

Heterozygous male mice (Cat^{Fr}/+ phenotype) obtained by crossing Cat^{Fr}/Cat^{Fr} females and BALB (+/+) males were used. The mice were kept in the Institute's vivarium on a conventional diet supplemented with greens throughout the experimental period. Cataract development was monitored microscopically. The lens was photographed in two projections once per month after dilating the pupil with 1% atropine. The severity of cataracts was graded from the photographs on a five-point scoring scale, assigning scores from 0 (undamaged lens) through 4 (total cataract) (Fig. 1). Animals were first allocated to subgroups according to the degree of lens opacification using a "right-and-left" comparison technique [1] and then randomly divided into a control and a test group, 10 mice in each. Mice of the test group were instilled with a 1% solution of catavidan (a water-soluble antioxidant

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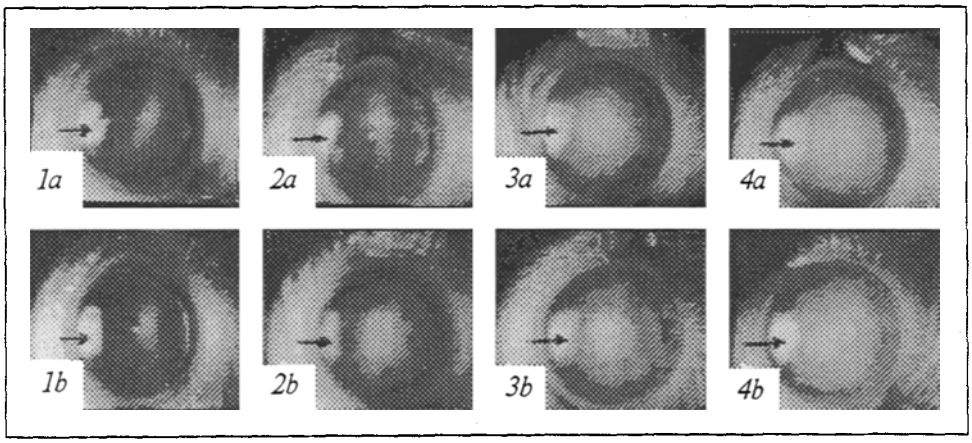

Fig. 1. Grades of lens damage in heterozygous Cat^{Fr} mice. *1a* and *1b*) slight diffuse opacification (score 1); *2a* and *2b*) diffuse opacity and small posterior polar cataract, respectively (score 2); *3a* and *3b*) posterior polar cataract (score 3); *4a* and *4b*) total cataract (score 4). Arrows indicate hot spots from the light guide of the photoflash.

from the class of sterically hindered phenols) into both eyes 5 times per week for 4 months, while the control mice were instilled with distilled water. The significance of differences between the two groups was determined using the two-tailed nonparametric Mann-Whitney test.

RESULTS

The experiment was started when the mice had reached the age of 2 months, and the appearances of cataracts in the control and test groups at that time are exemplified in Fig. 1. Two types of lens damage were seen: a diffuse opacity and a small posterior polar cataract. Cataract development proceeded through increases in the extent of diffuse opacification, with large numbers of swollen fibers appearing in the lens and progressively closing the posterior polar cataract.

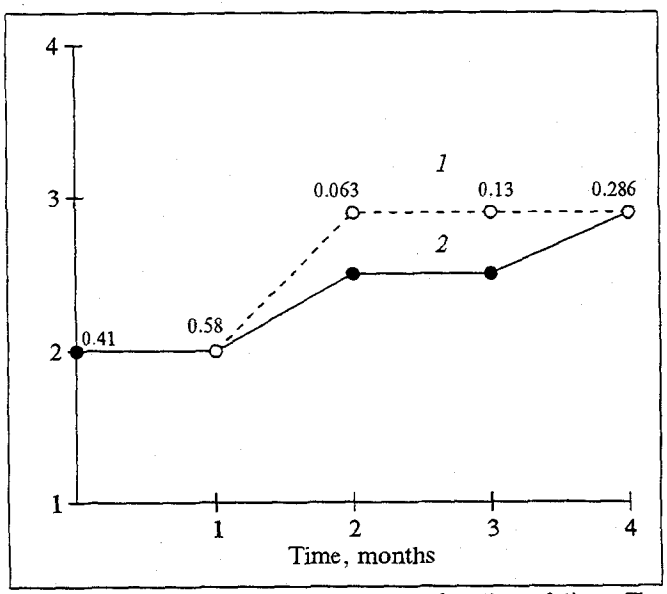

Fig. 2. Cataractous lens damage as a function of time. The ordinate shows median values for the control and test groups. *1*) control mice; *2*) test mice (instilled with 1% catavidan solution into both eyes). Figures represent the significance of differences, as estimated by the two-tailed nonparametric Mann-Whitney test, in the degree of lens damage between control and catavidan-treated heterozygous Cat^{Fr} mice.

After two months of catavidan treatment, the degree of lens damage in the test group was significantly less than in the control group (Fig. 2), and it could be seen that the process of fiber swelling was delayed in the treated animals. However, the differences between the test and control mice tapered off toward the end of the third month and disappeared almost completely by the end of the fourth. Mortality rates in the two groups were similar (5-8% per month).

Cataract formation involves a complicated chain of transformations that involves processes occurring both in the lens epithelium and in fully developed fiber cells. It has been suggested [2] that through the action of the Cat^{Fr} gene, the activity of one of the enzymes contained in the nuclear membranes of lenticular fibers is so altered as to impair the structure and function of the whole array enzymes mediating the transport of cytoplasmic and nuclear proteins that participate in DNA replication, in the regulation of synthetic processes, and in the formation of membranous cell components. The destructive processes taking place in the lens, such as vacuolation and the formation of defects in the nuclear membrane, most likely result from changes in the rates at which intracellular structures are assembled (synthesized) and disassembled. After a cataract had formed, for example, the relative content of γ-crystallins in the lens was found to be drastically reduced [7], and, as demonstrated earlier [4], FRO processes were greatly enhanced in cataractous lenses. Possibly, the enhanced FRO stimulates the activity of a high-molecular protease complex which has been shown to act specifically on oxidized proteins [6]. Also, the compartmentalization of Ca^{2+} may be disturbed, and this may activate the neutral protease calpain. According to David and Shearer, activation of proteases, including calpain, plays an

important part in cataractogenesis [5]. It is very likely that antioxidants can disrupt such an unfavourable course of events. It has recently been shown that antioxidants are also involved in cell regulation in other ways, in particular they are capable of protecting the cell from death as a result of a sharp fall in high-energy compounds consequent upon activation of poly(ADP-ribose)polymerase [8], and to be potentially implicated in transcription pathways by inhibiting activation of the nuclear factor NF-κB [9]. It should be noted, however, that the use of synthetic compounds such as antioxidants in a complex system like a living organism cannot be interpreted in a straightforward manner because synthetic antioxidants usually possess a number of properties that have nothing to do wiht their antioxidant properties - for instance, they are able to upset Ca^{2+} homeostasis in the cell by impairing cell membrane structure [3].

As the present study shows, the antioxidant treatment decreased the severity of cataractous lens damage only temporarily, and this can probably be taken as evidence that catavidan only acted upon and inhibited for a time some stage of cataractogenesis other than the key stage.

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