SYNCHRONOUS SCANNING STUDY OF FLUORESCENCE OF THE MOUSE LENS AT DIFFERENT STAGES OF X-RAY CATARACT

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Interest in the study of radiation-induced cataract is due to two factors: first, it is a frequent late after effect of radiation damage and, second, the development of radiation cataract, with respect to many parameters, resembles the development of senile cataract, the widespread distribution of which among old people is one of the most important problems in current ophthalmology [4]. Opacity of the lens in x-ray cataract is observed after a definite latent period [4]. Various changes have recently been found in the physical parameters of the lens before the development of opacity. However, essential changes in parameters such as a fall in the levels of thiols (TSH) in proteins [9], a rise of the temperature of phase separation of proteins [6], a rise of the monovalent cation level [11], and in the water content in the lens [10], take place immediately before the development of opacity. Meanwhile, at earlier stages corresponding to about half of the period until development of the ripe cataract, changes also have been found in some parameters, notably: a fall in the glutathione level (GSH, by about 30%) [9] and in the intensity of protein synthesis (by 40%) [8], coinciding in time with a fall of the K^+/Na^+ ratio. One of the characteristic features of the development of different types of cataract is the appearance of characteristic fluorophores, which may be either oxidation products of tryptophan (kynurenins) or lipid pigments of aging [1].

In the investigation described below the kinetics of accumulation of fluorescent products was studied at different stages of radiation-induced cataract in whole lenses, with simultaneous recording of the level of GSH, whose concentration is one of the earliest warning signs of cataract [9]. Since the recordable intensity of fluorescence of the lens depends on such parameters as its transparency, and orientation in the cuvette, to minimize errors of recording we used a method of synchronous scanning of fluorescence, in which the ratio between peaks of intensity of nontryptophan fluorescence and tryptophan fluorescence was used as the parameter. A similar approach was used previously to assess accumulation of aging pigments in a culture of nematodes [7], in cerebral cortical homogenates [2], and in homogenates of the calf lens [14].

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 mice were subjected to whole-body irradiation from a ^{60}Co source on the RKh-γ-30 apparatus (dose rate 8 rads/sec – 0.8 Gy/sec) in a dose of 5.00 Gy. The development of cataract was monitored by means of an opthalmoscope. Depending on the degree of development of radiation cataract, the lenses were classed in one of four stages [5]: I) the formation of separate punctate opacities in the lens; II) punctate opacities fusing together to form a small opaque disk in the center of the posterior subcapsular region; III) the disk has increased in size and rays are spreading from it toward the periphery; IV) total opacity of the lens. As the control, transparent lenses of unirradiated animals of the same age were used. The lenses were extracted immediately before the experiment. Parameters of fluorescence of the lenses were measured immediately after extraction on a "Hitachi-MPF4" fluorometer, at a right angle, at room temperature, in 0.14 M NaCl, 0.01 M Tris-HCl buffer, pH 7.4. To record fluorescence we used a special quartz cuvette with cylindrical support, covered with black paper to reduce scatter of the exciting light. TSH (total SH + S-S groups) and

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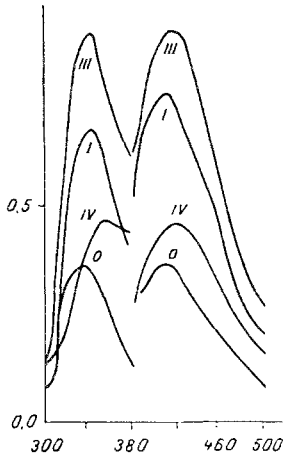

Fig. 1

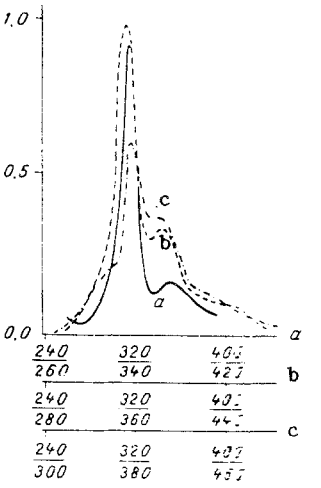

Fig. 2

Fig. 1. Excitation (λ_{fl} = 420 nm) and emission (λ_{em} = 345 nm) spectra of nontryptophan fluorescence of whole lenses of mice with different stages of radiation cataract. I-IV) Stages of development of radiation cataract. 0) Unirradiated animals.

Fig. 2. Synchronized scanning spectra of whole lenses of mice with different fixed difference of wavelengths (stage I radiation cataract). Formative (Fig. 3b). Fixed difference of wavelengths: a) 20 nm, b) 40 nm, c) 60 nm. I) Intensity of fluorescence (in relative units).

TABLE 1. Changes in Concentrations of Thiols (TSH) and Glutathione (GSH) and Ratio of Peaks of Fluorescence (K) in Synchronized Scanning Spectra of CBA Mouse Lenses at Different Stages of Radiation Cataract

Parameter	Unirradiated animals	Stage			
		I	II	III	IV
TSH, mmoles/mg tissue	62,2±6,7	57,8±7,3	62,5±2,2	67,9±0.6	32,2±4.5
GSH, mmoles/mg tissue	33,9±0,8	7.00±0.6	5,90±0,8	5,6±0.8	5,6±0.8
K, relative units	0,03±0,01	0.11±0.02	0,11±0,01	0,09±0,01	0,08±0,01

GSH were determined by the method in [13]. The lenses, previously weighed, were homogenized in 8.0 ml of 0.02 M EDTA. Next, 0.5 ml of homogenate was taken for estimation of thiols and mixed with 1.4 ml of 0.2 M Tris-HCl buffer, pH 8.2, with the addition of 0.1 ml of 0.01 M Ellman's reagent and 1.0 ml of methanol. After sedimentation of the proteins by centrifugation (3000g, 15 min) absorbance of the supernatant was measured at λ = 412 nm. To determine GSH, 4.0 ml of distilled water and 1.0 ml of 50% TCA were added to 5.0 ml of lens tissue homogenate. The tubes were vigorously shaken and the contents centrifuged for 15 min at 3000g. Next, to 2.0 ml of supernatant were added 4.0 ml of 0.4 M Tris-HCl (pH 8.9) and 0.1 ml of 0.01 M Ellman's reagent. Absorbance was measured at 412 nm. The GSH concentration was determined by reference to calibration graphs, using reduced GSH (Reanal, Hungary). The GSH concentration was expressed per milligram wet weight of lens.

EXPERIMENTAL RESULTS

One of the manifestations of senile cataract is the appearance of characteristic fluorescence of the lens with maximum of emission in the 420 nm region [12]. This fluorescence is known to be connected with the appearance of oxidized forms of protein tryptophanyls, and in particular, of kynurenins [4]. In the lenses of the irradiated mice, a significant increase in nontryptophan fluorescence took place even in the first stage of radiation cataract, with a characteristic maximum of excitation at 345 nm and of emission at 420 nm (Fig. 1).

However, the direct use of the intensity of nontryptophan fluorescence as marker of the development of radiation cataract is complicated by the strong dependence of fluorescence

on the position and orientation of the lens in the cuvette. Accordingly we chose the method of synchronized scanning, in which the ratio between peaks of intensity of nontryptophan and tryptophan fluorescence is used as the parameter. This parameter is relatively insensitive to the position of the lens in the cuvette, for with different arbitrary positions of the lens its variation amounts to under 8%, whereas variation of intensity itself may be by a factor of more than 1.5 times.

The synchronized scanning method consists of recording fluorescence during synchronized scanning of excitation and emission monochromators with fixed difference of wavelengths [3]. The shapes of the synchronized scanning spectra depend essentially on the shift between the wavelengths of emitted and exciting light. Typical synchronized scanning fluorescence spectra of the lens with different fixed wavelength differences are illustrated in Fig. 2. The clearest separation of the fluorescence peaks of the fluorophores is observed when the difference between wavelengths is 20 nm. This difference of wavelengths also is recommended in [3] and [14]. Subsequently, to record synchronized scanning spectra, a shift of 20 nm was used. The change in the value of the peak ratio ($K = I_{360/380}:I_{320/340}$) at different stages is shown in Table 1. It will be clear from these data that even in the first stage, when only scattered punctate opacities could be detected, the value of K was increased by 3.5 times. During development of the cataract (stages II and III) the increase in K was small. However, the decrease in K at stage IV may be attributable to protein destruction and elution of peptides from the lens, evidence in support of which is given by the fall in the TSH concentration in stage IV.

The distinct correlation between the ratio of peaks of intensity in the synchronized scanning spectra and the GSH level in the lens will be noted. According to [9], a fall of its level is one of the earliest warning signs of changes leading to the development of radiation cataract. Correlation between the parameter K and the fall of GSH suggests that recording synchronized scanning spectra may be an informative indicator of the development of radiation cataract at pre-opacity stages. The absence of any significant changes in the TSH concentration in the first three stages of development of radiation cataract is in good agreement with the results of a study of radiation cataract in rabbits, in which the TSH level in the rabbit lens fell significantly only 1 week before the development of a ripe cataract [9].

The parameter K, which changes significantly even in the early stage of development of radiation cataract and which correlates with the GSH concentration in the lens, is therefore a highly sensitive and informative parameter of cataract formation

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