

Senile Cataract and the Absorption Activity of Cytochrome C Oxidase

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ABSTRACT

The aim of the study was to determine the activity of cytochrome c oxidase in mitochondrial fractions of cataractogenic epithelial cells of lenses of 60 patients (34 females and 26 males). According to clinical criteria patients were divided into three groups: group 1 – patients with senile cataract, group 2 – active smokers with senile cataract, and group 3 – diabetic patients with cataract as diabetic complication. In the extracted lenses we determined the absorption activity of mitochondrial enzyme cytochrome c oxidase. Biochemical researches using the method of Yonetani and Ray¹³ were applied. We also applied the statistical Student t-test ($p < 0.05$) and the variance analysis (R. Fisher) with three parameters and Snedecor F distribution ($s = p < 0.001$). The activity of cytochrome c oxidase in mitochondrial fraction of lens epithelial cells is twice as low in patients who are active smokers than in the non-smoking patients with senile cataract. We measured a significantly different activity of cytochrome c oxidase between active smokers and the non-smoking patients with senile diabetic cataract ($tp < 0.05$, $sp < 0.001$). Cigarette smoke decreases the activity of cytochrome c oxidase in mitochondrial fraction of lens epithelial cells. This enzyme is a terminal oxidase in the synthesis of ATPs. We suggest that smoking decreases the synthesis of energy in the lens of cigarette smokers. This emphasizes the significance of giving up smoking in order to preserve the structure and function of the lens.

Introduction

Eyes are permanently exposed to UV rays¹, changes of pH values², and various metabolites^{3,4}. These processes generate reactive free radicals⁴⁻⁶. The most fre-

quent radicals are hydroxide radicals (OH) and superoxide radicals (O_2)^{2,5,6}. Free radicals include reactive oxygen species with unmatched electrons in their electronic layer. Their oxidation damages different macromolecules in cells, includ-

ing DNA, proteins and lipids^{2,4,6,7}. Free radicals in the lens accumulate opaque crystallins in browning agglomerates^{1,3,5}.

Diabetes is an example of biochemical changes that cause denaturation of proteins in mitochondrial and microsome epithelial cells^{4,7–11}. The lens becomes blurred and the eye develops cataract^{1–3,5–7,11}. The anatomy, microscopic structure, physiology of the lens, clinical picture of the cataract and surgical treatment are all well known, but cataractogenesis has not been studied enough^{7–12}.

The aim of the research was to determine the influence of smoking on the development of cataract in active cigarette smokers, by means of biochemical procedures for measuring the concentration of substances and enzymic activity of cell organelles in blurred lenses.

Patients and Methods

According to relevant clinical criteria we divided patients with blurred lenses into three groups: Group 1 included old people with senile cataract. They were non-smokers, did not have diabetes and were physically ready for operation. Included were extracapsularly extracted lenses of 105 patients (67f and 38m) with impaired vision. The mean age was 71.6. Group 2, experimental group, included patients who were active cigarette smokers with senile cataract. They were in their early and middle old ages. They were healthy, did not have diabetes and were clinically ready for cataract surgery. Included were extracapsularly extracted lenses of 65 patients (13f and 52m) with impaired vision. The mean age was 63.6 years. Group 3, experimental group, included diabetic patients with senile cataract. They were verified insulin dependent and independent, regulated diabetic patients with blurred lenses and normal HbA_{1c} below 7%. They did not show extracapsular complications. They had normal diabetologic findings for cataract

surgery. Included were extracapsularly extracted lenses of 45 patients (32f and 13m) with impaired vision. The mean age was 73.9 years.

The extracapsularly extracted cataracts were biochemically analyzed. Because of the complicated experimental process a sample of frozen lenses (60; 20 in examined groups) was randomly chosen. Extracted cataracts were homogenized in 0.1 M phosphate buffer (pH 7.5) and homogenized at 1000 × g for 10 minutes. The obtained supernatant was centrifuged again at 10000 × g for 30 minutes and final mitochondrial pellet was solubilized in phosphate buffer (pH 7.5). The activity of mitochondrial enzyme cytochrome *c* oxidase was determined using spectrophotometer, Unicam SP 500 at 550 nm, by the method of Yonetani and Ray¹³. Cytochrome *c* was reduced by sodium dithionit to ferrocytochrome *c*. Cytochrome *c* oxidase oxidized ferrocytochrome, which changed the absorbency of the sample at 550 nm. The difference of absorption was reduced and the oxidized form was calculated as the specific activity¹³.

The activity of cytochrome *c* oxidase is expressed in nmol/mg protein/min. The concentration of proteins was determined by the method of Lowry et al.¹⁴, using bovine serum albumin as standard.

The achieved results were statistically analyzed by the variant analysis with three parameters (R. Fischer) and tested with the Snedecor-F-distribution test ($p < 0.001$) and Student t-distribution test ($p < 0.05$).

Results

Among 215 patients there were 112 (52%) females and 103 (48%) males. The mean age was 69.7. There were 11 (18.4%) patients younger than 56 years, 7 (11.1%) of them were smokers with cataract, from the group with the mean age of 63.6. By comparing the mean ages of the groups we can see that active smokers

TABLE 1
SPECIFIC ACTIVITY OF CYTOCHROME C OXIDASE IN MITOCHONDRIAL FRACTION OF
EPITHELIAL CELLS OF BLURRED LENSES IN EXAMINED PATIENTS

Groups of patients	Cytochrome <i>c</i> oxidase		
	n	nmol/mg proteins/min	p
Senile cataract	20	4.81 ± 0.42	< 0.001
Cataract in smokers	20	2.47 ± 0.19 s,s ₁	< 0.001
Cataract in diabetic patients	20	6.76 ± 0.61	< 0.001

s – compared to the group of patients with senile cataract, $F = 0.170$

s₁ – compared to the group of diabetic patients with cataract, $F = 0.179$

t = 2.86sd, $p < 0.05$

with senile cataract were younger than other non-smoking patients with cataract.

We measured a specific activity of cytochrome *c* in mitochondrial fraction of epithelial cells of blurred lenses in a sample of 60 patients, 20 patients in each group, by means of the above-mentioned methods. The lowest measured activity of cytochrome *c* was 2.47 nmol/mg proteins/min in the group of smoking patients with senile cataract. In this group the activity was twice as low compared to other groups of patients. According to that, we found a statistically significant difference between the activities of cytochrome *c* oxidase in these groups ($s = p < 0.001$) (Table 1).

Discussion

Cigarette smoke comprises many harmful compounds which damage the function of the lens^{1,3,14–16}. In our experiment, at the time of cataract extraction smoking patients were 8 to 10 years younger than other non-smoking patients. We found that the activity of cytochrome *c* oxidase is low (2.47 nmol/mg proteins/min) in mitochondrial fraction of epithelial cells of blurred lenses in patients who smoke and that it is twice as low compared to the corresponding values of the same fractions in other patients.

The lens is an encapsulated organ without blood vessels that requires a great quantity of ATPs for diffusion of different molecules and metabolites from ciliary body to the lens^{17,18}. Ocular structures are rich with enzymes whose activities are better examined in aqueous, retina and vitreous than in the lens^{17,18}.

Cytochrome *c* oxidase presents the terminal enzymic complex in a respiratory chain of mitochondria and it is an essential compound for the synthesis of ATPs^{12,13,19}. This enzyme reacts with oxygen and prevents the development of aggressive superoxide radical¹⁹.

Cytochrome *c* oxidase is identical to Warburg's respiratory enzyme. When it is in a reduced form it is recognizable by its clear absorption spectrum (min 450nm, max 590 nm). For oxidation of cytochrome *c* we need cytochrome oxidase. Way found that the activity of cytochrome *c* oxidase is inhibited by cyanide which is a toxic compound of cigarette smoke²⁰.

The lower quantity of ATPs in epithelial cells of smokers can act as a biochemical factor during cataractogenesis. This explains the fact that the mature cataract in smokers appears 8 to 10 years earlier than in non-smokers with senile cataract.

Conclusion

Active smoking (> 5 years, 20 cigarettes a day) accelerates the development

of cataract. Our results, which confirm that smoking is an essential factor in the development of cataract, correspond to the results of West at all.¹⁵

Mitochondrial enzyme of cytochrome c oxidase, which participates in the synthesis of ATPs, shows low specific activity in epithelial cells of blurred lenses in smokers (2.47 nmol/mg proteins/min), and it is twice as low compared to the same frac-

tions of epithelial cells in non-smoking patients.

These results indicate the possibility that smoking has harmful effect on the eye lens and that it participates in the development of cataract. This emphasizes the significance of giving up smoking in order to preserve the structure and function of the lens.

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OČNA KATARAKTA I AKTIVNOST CITOKROM C OKSIDAZE

SAŽETAK

Katarakta nastaje zbog promjena kristalina u leći. Dim iz cigareta mijenja strukturu, morfologiju i funkciju leće. U radu je određivana aktivnost citokroma c oksidaze u mitohondrijima kataraktogenih epitelnih stanica leća u 60 ispitanika (34ž, 26m). Prema kliničkim kriterijima bolesnici su razvrstani u tri skupine: 1 – bolesnici sa senilnom kataraktom, 2 – bolesnici s kataraktom pušača i 3 – bolesnici s dijabetičkom kataraktom. Proveli smo biokemijska ispitivanja metodama po Wahrtonu¹³ i Rayu¹⁵. Ustanovili smo da je aktivnost enzima citokrom c oksidaze niska (2,47 nmol/mg proteina/min) u mitohondrijskoj frakciji bolesnika s kataraktom pušača, a dvostruko manja u istim frakcijama epitelnih stanica nepušača.