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Professional paper

Protein Carbonylation and Glycation in Human Lenses

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A B S T R A C T

It has been observed protein carbonylation and glycation in the lens epithelial cell fractions of lenses in people with mature cataract. We used lenses of diabetic patients, weak and strong cigarette smokers and people who had senile cataract. The protein glycation is the highest in a diabetic senile cataract patients and the lowest in non-diabetic senile cataract patients. The protein carbonylation is extremely high in mitochondrial epithelial cells of the strong cigarette smokers. These results show that the glycation and carbonylation of the human lens proteins are the part of the cataract development in people. Specific factors, such as high glucose in diabetes and the tobacco smoke (in cigarette smokers), can change the lens structure, thus stimulating cataract development.

Introduction

Lens is a transparent structure, located between iris and vitreal body in a posterior chamber of the eye. The lens consists of the inner nucleus (the core) and the outer cortex [1,2]. The core contains epithelial cells, which are developed yet in the embrional life. The lens capsule is formed by elongated epithelial cells, the fibrous lens fibers that lie in the equatorial zone. The eye is constantly exposed to UV light, heat, pH changes, various metabolites, age, and free radicals. Their interaction leads to cataract formation. In the last few years it has been proved that free radicals are the most important factor in damage progression of lens and retina.

Free radicals are the numerous oxygen joints, which have unpaired electrons in their electronic sheet [2,3]. The big molecules such as DNA, proteins, carbohydrates and lipids are very easily oxidated by those radicals [2–4]. The eye is constantly exposed to UV rays, which are the important factor in a cataract development[2–4]. Accumulation of the lens protein-crystalline, in big agglomerates [1,2,4,5] leads to development of the cataract. In those processes crystalline glyacation and carbo-

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nylation may occur. Glycation is a non-enzymatic condensation of the lysine terminal groups with sugar / glucose. The product set in that way is a stable chemical joining called Amadori's product[6]. Carbonylation is decomposition between carbon and nitrogen inside the aminoacid remains, which leads to release of the carbonyl products[7,8]. Therefore, many proteins lose their biological function because of the carbonylation. The measurement of the carbonylation became a good indicator of the oxidative damage of numerous proteins in the human body[2,7,8].

The aim of the experiment is the measuring of the carbonylation and glycation in the organelles of the lens epithelial cells in people with mature cataract.

Materials and Methods

The lenses are gotten after the cataract surgery, from the Department of Ophthalmology, Clinical hospital Osijek. There were 20 patients with senile cataract, 9 with complicated diabetic cataract, and 18 patients who were cigarette smokers for many years, 11 of them weak and 7 strong (hard) smokers (more than 20 cigarettes per day during at last 5 years). All the patients were between 58 and 75 years old. The lenses taken after the surgical treatment were homogenized with 1.0 ml of phosphate buffer pH 7,5 and centrifuged 10 minutes at 1000 × G to get insoluble protein fraction. The supernatant is than centrifuged again at 15000 × G, 30 minutes to get mitochondrial sediment and post-mitochondrial supernatant. The mitochondria were diluted in a small amount of phosphate buffer pH 7.5 and frozen at –20 C till further use.

Protein glycation of the mitochondrial sediment and the post-mitochondrial supernatant fraction from the lens epithelial cells was determined by the method of Ahmed and Furth[9]. By this technique glycated proteins make colored complex with periodate and acetylacetion, which can be measured in spectrophotometer by 405 nm. Carbonyl proteins make a yellow complex with 2.4 dinitrophenylhydrazin. The intensity of that color is measured by 370 nm in spectrophotometer. The protein concentration in measured samples was determined by the method of Lowry and Co. [11]. The statistic difference degree between tested groups is determined by variance analysis.

Results

The protein glycation and carbonylation in the lens epithelial cell fractions is shown in Figures 1 and 2. The protein glycation is 10 times greater in the mitochondrial fraction in contrast to the post-mitochondrial fraction (Figure 1). In both organelles the biggest carbonylation level is measured in the lenses of diabetic patients. The protein carbonylation of the lens cell fraction is shown in nm/mg proteins and demonstrates different distribution. The highest level of carbonylation in the mitochondrial fraction is in the lenses of the strong smokers while in post-mitochondrial fraction is in the lenses with senile cataract (Figure 2.).

The carbonylation / glycation ratio is shown in the Table 1. The biggest differences between the organelles are between the mitochondria of the active smokers and the post-mitochondria of the people with senile cataract. In the same time the smallest differences are between the cell fractions in diabetic patients.

Discussion

Around 90 % of the lens dry matter are the proteins-crystalline [1,210,12]. During our life the proteins change very slowly. The crystalline are exposed to UV rays, free radicals etc. all our life. The lens transparence is turning to the cata-

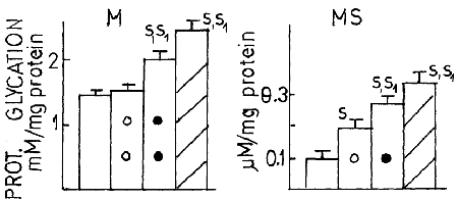

Fig. 1. Protein glycation of lens epithelial mitochondrial (M) and post-mitochondrial (MS) fraction. Groups: senile cataracts, smokers less then 20 cigarettes per day (open circles), smokers over 20 cigarettes per day (drawn circles), diabetic patients (partially drawn circles).

s = p < 0.01 to senile cataract patients
s1= p < 0.01 to smokers < 20 cigarettes per day

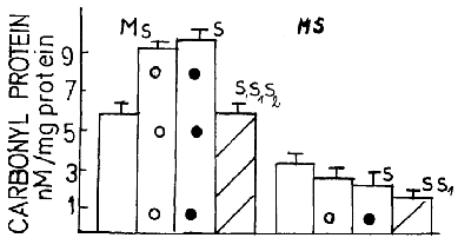

Fig. 2. Protein carbonylation of lens epithelial mitochondrial (M) and post-mitochondrial (MS) fraction. Groups: senile cataracts, smokers less than 20 cigarettes per day (open circles), smokers over 20 cigarettes per day (drawn circles), diabetic patients (partially drawn circles).

s = p < 0.01 to senile cataract patients
s1= p < 0.01 to smokers < 20 cigarettes per day
s2= p < 0.01 to smokers > 20 cigarettes per day

ract because of the sedimentation and accumulation of the crystalline into bigger protein agglomerates[2,10]. These examinations show that there is a great level of glycation and carbonylation in the lens epithelial cells fractions. The glycation is very high in the lenses of diabetic patients, because of the high plasma glucose level. This process is 40% higher than glycation in the lenses with senile cataract. The protein glycation is important factor in development of diabetic complications[12]. These results may lead to possibility that the protein glycation in diabetic patients enhance the cataract development. The lens protein carbonylation is extremely high in people who were high smokers for many years. These results correlate with those of Christiansen[13] and West and Co. [14] which show that the smoking enhances the cataract development. The tobacco smoke consists of almost 4000 inorganic and organic substances[15] and some of them can influence the crystalline structure of the lens in the active cigarette smokers. In conclusion we can say that the lens protein glycation and carbonylation is the important part of the cataract development process. By diabetic patients, a key factor is, of course, the glucose high level and by smokers, components of tobacco smoke. Smoking cigarettes, as the important risk factor and the cause of many chronic diseases has an important role in the cataract development.

TABLE 1
RELATION BETWEEN CARBONYLATION AND GLYCATION OF MITOCHONDRIAL (M) AND POST-MITOCHONDRIAL (MS) FRACTION OF HUMAN LENS EPITHELIAL CELLS

Cataract type	M	MS
senile	4.23	35.61
smokers (20 cig. per day)	6.40	12.39
smokers (20 cig. per day)	5.00	10.59
diabetic	2.52	5.87

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KARBONILACIJA I GLIKACIJA PROTEINA U LEĆAMA LJUDI

SAŽETAK

Promatrana je glikacija i karbonilacija proteina u frakcijama epitelnih stanica leća ljudi s razvijenom kataraktom. Promatrane su leće dijabetičara, slabih i jakih pušača cigareta, te ljudi sa senilnom (staračkom) kataraktom. Glikacija proteina je najveća u organelima epitelnih stanica leće i najmanja kod leća u ljudi sa staračkom kataraktom. Karbonilacija proteina leće je izrazito visoka u mitohondrijima epitelnih stanica jakih pušača cigareta. Ovi rezultati pokazuju da su glikacija i karbonilacija proteina leća sastavni dio procesa razvoja katarakte u ljudi. Specifični štetni čimbenici kao što su glukoza i duhanski dim cigareta kod pušača mogu promijeniti strukturu proteina leće i time stimulirati razvoj katarakte.