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e-mail: havvita@ukr.netASSESSMENT OF HUMAN HAIR DAMAGE
AFTER OXIDATIVE STRESS

Key words: human hair, treatment, protein loss, solubilized melanin, alkali solubility

Abstract. The paper presents the results that characterize the degree of human hair damage after oxidative stress. It was established that changes in the chemical structure of the hair, induced by oxidative stress depended on the duration of the damaging factor exposure. It has been shown that due to splitting of disulfide bonds in keratins elution of protein from hair fibers raises increasing the amount of solubilized melanin and the alkali solubility of the fiber. The most characteristic changes in hair composition was observed after a 90 minute treatment.

Introduction

Recently the interest in hair biology studying due to significant advances in cosmetic industry has been increased. According to the expert's opinion the world market of hair care products is estimated over 26 billion [4]. Therefore, the fundamental understanding of the hair structure, its composition and properties is essential.

It is known that human hair can be divided into the following structural components, as the cuticle, cortex and medulla.

Medulla is the centermost part of the hair. It is composed of loosely packed keratinized cells. Cortex is formed by elongated spindle cells, which were surrounded by cell membrane complex. Cortical cells also contain melanin granules. Hair pigments protect the proteins from photo degradation [8].

The outermost layer is cuticle which forms the surface of the hair fibre and surrounds the fibre bundles of the cortex cells. The cuticle offers protection to the internal components of the fibre from mechanical and chemical weathering. It consists of flat overlapping cells. There are between 5-10 layers of cuticle cells in human hair.

Thus, hair is a subject of influence of various factors that lead to impair of its properties [2, 5, 7, 10]. The chemical agents that can damage the hair fiber include bleaching, dyeing and perming. Frequent use of chemical agents is a major cause of hair shaft damage that is accompanied by morphological changes on the hair surface and alteration of its composition. Physical causes of hair shaft damage include friction, photo damage and daily grooming. Exposure to sunlight and ultraviolet radiation lead to dryness, rough surface texture, decreasing of colour and luster and increasing of stiffness and brittleness.

The aim of the study

To design the oxidation stress by chemical treatment and analyses of the changes in biochemical

composition of the fiber. The better understanding of interaction between fiber and damaging agent might be useful for developing new cosmetic products. Such information may also be important for understanding the changes that occur in different animal fibers after various treatments.

Materials and methods

Chemically untreated dark brown hairs were collected from females. The length of hair samples was about 10 cm and each bunch about 5 g in weight. Average hair diameter was about 70 μ m. Hair samples were soaked in 1 % SDS (w/v) and then washed with de-ionized water and air dried. Oxidative treatment was performed by sample immersion into bleaching solution with a liquid to fiber ratio of 50:1 at 37°C for 30, 60, 90 min. Bleaching solution was prepared by mixing of solution A and solution B in equal proportions. Solution A contained 10 g urea, 7 g NaCl, 12 ml ammonia (28 %) diluted in 100 ml de-ionized water. Solution B (6 % H₂O₂) contained 17,1 ml H₂O₂ and 82,9 ml de-ionized water. The treated hair samples were rinsed with de-ionized water and dried at room temperature. The different groups of samples such as intact human hair and hair treated with bleaching solution were compared.

All reagents were of analytical grade.

For the assessment of protein loss in the hair 0,2 g hair samples (2 mm length) were added to falcon tubes [11]. Distilled water was added at a ratio of 10:1 (water to hair) and shaken at 37 °C on the shaker during 24 hr. Then water extract was removed from tubes and protein concentration in the extracts from the human hairs was determined according to a protocol, based on the colorimetric quantification [3]. Protein concentrations were calculated using the standard curve of bovine serum albumin. Melanin concentrations were determined by hydrolysis in 0.85 M KOH at 100°C, followed by measurement of absorbance at 400 nm and extrapolation from a cal-

ibration curve, which were obtained with melanin [6].

Alkali-solubility test was performed according to [1]. The hair samples were treated with a solution of 0,1 M NaOH and the differences between the dry weight of the samples before and after the treatment were determined. The percentage of alkali solubility was calculated.

The t-test was used to determine whether differences between untreated and treated hair were statistically significant.

Results and discussion

The results about protein loss in the hair after oxidation stress are presented in Fig.1.

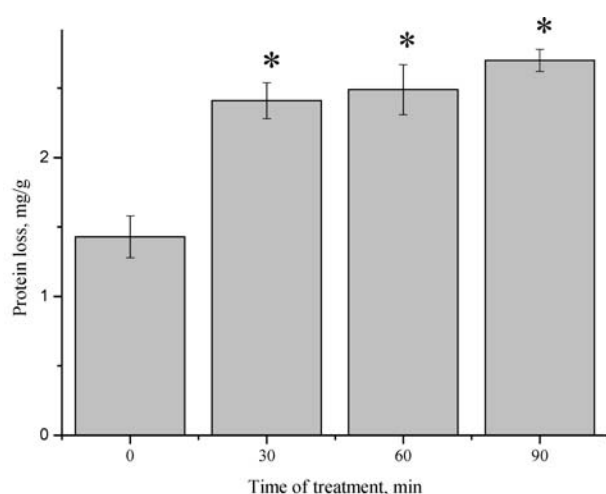


Fig. 1 Protein loss after hair treatment (expressed in mg/g of hair, $M \pm m$, $n=5$), * - the differences between treated and untreated hair are statistically significant ($P \leq 0,05-0,001$)

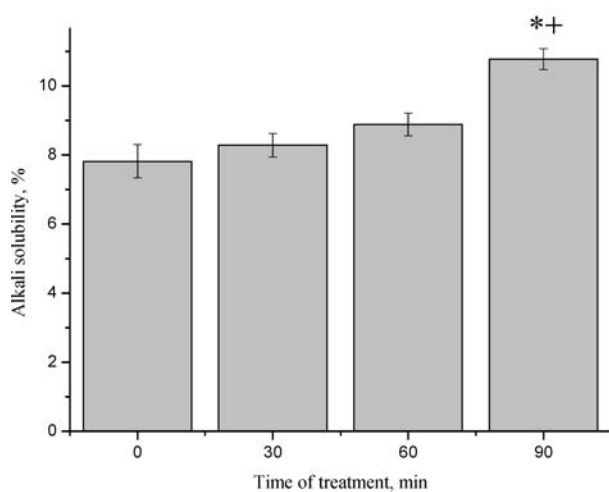


Fig. 2 Alkali solubility of hair after treatment (expressed in percentage of fiber solubility, $M \pm m$, $n=5$), * — the differences between treated and untreated hair are statistically significant ($P \leq 0,001$), + — the differences between treated hair during 60 min and treated hair during 90 min are statistically significant ($P \leq 0,01$)

The remarkable differences between untreated and treated hair were observed. Thus, the protein loss in the hair after oxidative treatment increased more than 1,6 times ($P \leq 0,01-0,001$). It should be noted that the changes of protein loss in the hair due to the time of treatment were small.

Alkaline oxidizing composition, which includes hydrogen peroxide, induces the breakage of S-S bond. Moreover, an alkaline component in the bleaching solution causes a scission of peptide bond of main chain and disappearance of ionic bond between chains. The hydrolytic attack on the amide bonds is accompanied by elution of proteins from hair fiber [9].

Fig. 2 shows the results, which are obtained after alkali solubility test. The alkali solubility is sensitive marker of fiber damage and is widely used for evaluation of wool. This test indicates the hydrolysis of cystine — an amino acid which is extremely important for the formation of mechanical properties of human hair.

It was established that a low value of alkali solubility was found in untreated human hair. The data show that chemical treatment is reflected in an increase of alkali solubility of hair but there was no significant difference between untreated and bleached hair during 30 and 60 min. A maximum of alkali solubility of about 11 % ($P \leq 0,02$) was observed in bleached hair after 90 min of treatment.

The changes in solubilized melanin after oxidative treatment are illustrated in Fig 3.

After treatment for 30 and 60 min the amount of solubilized melanin tended to be greater than in untreated hair. Bleaching of hair for 90 min is charac-

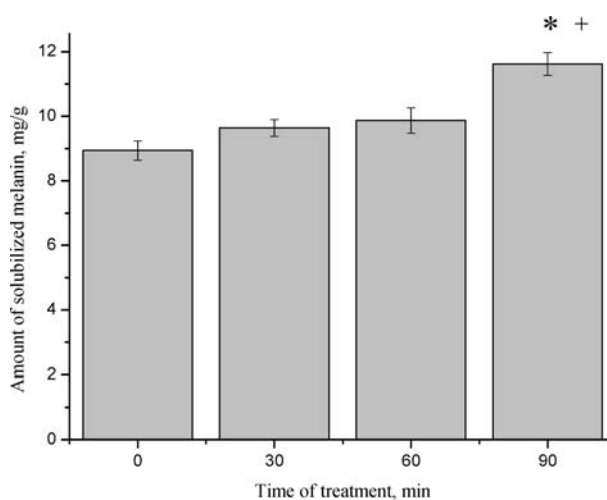


Fig. 3. Amount of solubilized melanin after hair treatment (expressed in mg/g of hair, $M \pm m$, $n=5$); * — the differences between treated and untreated hair are statistically significant ($P \leq 0,001$), + — the differences between treated hair during 60 min and treated hair during 90 min are statistically significant ($P \leq 0,01$)

terized by significant increase of solubilized melanin content. Similar data were observed by Takada K. and co-workers [10].

Conclusion

We conclude that changes in the chemical structure of the hair, induced by oxidation stress depend on the duration of the damaging factor exposure. Chemical treatment of hair is accompanied by the elution of protein from hair fibers, increasing of the amount of solubilized melanin and the alkali solubility of the fiber. The most characteristic change in hair composition was observed after a 90 minute treatment.

Perspectives

The effect of oxidation stress on the some amino acids content in the hair will be further investigated.

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ОЦІНЮВАННЯ СТУПЕНЯ ПОШКОДЖЕННЯ ВОЛОСА ЛЮДИНИ ЗА ДІЇ ОКСИДАТИВНОГО СТРЕСУ

В. В. Гавриляк

Резюме. Волос є об'єктом впливу різноманітних факторів, що призводять до змін його структури та властивостей. До чинників, здатних пошкоджувати волос, належать фарбування, відбілювання, нагрівання, тертя, ультрафіолетове опромінювання. Мета досліджень полягала в моделюванні оксидативного стресу та аналізі змін, що відбуваються у структурі волоса. В експерименті використовували два типи зразків волосся, які умовно називали хімічно оброблені та необроблені. Оксидативний стрес моделювали шляхом хімічної обробки волоса за допомогою окисників. Оцінювали втрату протеїну із стержня волоса, його розчинність та вміст меланіну. Встановлено, що зміни в хімічній структурі волоса, індуковані оксидативним стресом, залежать від тривалості впливу пошкоджуючого чинника. Показано, що внаслідок розщеплення дисульфідних зв'язків у кератині підвищується елюція протеїну з волоса, збільшується його розчинність у лузі та зростає кількість розчинного меланіну. Найхарактерніші зміни спостерігали після 90 хвилинної обробки волоса.

Ключові слова: волос людини, обробка, втрата протеїну, розчинний меланін, розчинність у лузі

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ОЦЕНКА СТЕПЕНИ ПОВРЕЖДЕНИЯ ВОЛОСА ЧЕЛОВЕКА ПОД ВЛИЯНИЕМ ОКСИДАТИВНОГО СТРЕССА

В. В. Гавриляк

Резюме. В статье представлены результаты, характеризующие степень повреждения волоса человека под влиянием оксидативного стресса. Сделан вывод, что изменения в химической структуре волоса, индуцированные оксидативным стрессом, зависят от длительности воздействия повреждающего фактора. Показано, что в результате расщепления дисульфидных связей кератина повышается элюция протеина из волокна, увеличивается растворимость волокна и количество растворимого меланина. Характерные изменения наблюдали после 90 минутной обработки волоса.

Ключевые слова: волос человека, обработка, потеря протеина, растворимый меланин, растворимость в щелочи.

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