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CORRELATION OF NUCLEOTIDES AND CARBOHYDRATES METABOLISM WITH PRO-OXIDANT AND ANTIOXIDANT SYSTEMS OF ERYTHROCYTES DEPENDING ON AGE IN PATIENTS WITH COLORECTAL CANCER

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Aim: **To examine the relationship between metabolic features of purine nucleotides and antioxidant system depending on the age of patients with colorectal cancer.** ***Materials and Methods*****: The activity of adenosine deaminase, xanthine oxidase, glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase, the NOx concentration and the oxidative modification of proteins were determined spectrophotometricaly in 50 apparently healthy people and 26 patients with colorectal cancer stage III–IV, aged 40 to 79 years.** ***Results:*** **Increase of pro-oxidant system of erythrocytes with the age against decrease in level of antioxidant protection in both healthy individuals and colorectal cancer patients was determined. A significant increase of products of oxidative proteins modification in erythrocytes with ageing was shown. Statistically significant correlation between enzymatic and non enzymatic markers pro-oxidant system and the activity of antioxidant defense enzymes in erythrocytes of patient with colorectal cancer was determined.** ***Conclusion:*** **Obtained results have demonstrated the imbalance in the antioxidant system of erythrocytes in colorectal cancer patients that improve the survival of cancer cells that is more distinctly manifested in ageing.**
Key Words: **age, colorectal cancer, erythrocyte, nucleotides, antioxidants, oxidative stress.**

It is known that ageing is accompanied by the deterioration of protective functions of the organism and weakening of antioxidant defense (AOD). The metabolism of carbohydrates, proteins and nucleotides is underwent changes, in particular amplification of catabolic processes is observed resulting in the accumulation of toxic substances, including carcinogens, that is promoted by reactive oxygen species (ROS) [1, 2]. Mentioned processes are significantly amplified with ageing that indicates the necessity to concentrate the attention on the peculiarities of tumor growth in older patients.

Colorectal cancer (CC) — is a widespread pathology in the world; among the CIS countries the highest incidence of disease is observed in Ukraine [3]. Number of patients suffering from CC is growing steadily. This type of cancer takes one of the first places on mortality in Cancer Registry among men and women [4]. Among the methods for cancer treatment, surgery supplemented with chemotherapeutic drugs [5] remains currently the main one. But the question of the use of antioxidants and pro-oxidants in the treatment of oncology patients has not been fully explored [6].

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Abbreviations used: ADA – adenosine deaminase; ADNPH – aldehyde dinitrophenylhydrazones; AOD – antioxidant defense; AOS – antioxidant system; CC – colorectal cancer; G6PDH – glucose-6-phosphate dehydrogenase; GPO – glutathione peroxidase; KDNPH – ketone dinitrophenylhydrazones; NO – nitric oxide; OMP – oxidative modification of proteins; $ONOO^-$ – peroxynitrite; POS – pro-oxidant system; PPP – pentose phosphate pathway; ROS – reactive oxygen species; SOD – superoxide dismutase; XO – xanthine oxidase.

The objective of this work is to determine the activity of key enzymes of purine nucleotides decomposition — adenosine deaminase (ADA) and xanthine oxidase (XO), as sources of ROS formation [7] (Figure), as well as non-enzymic representative of pro-oxidant system (POS) — nitric oxide (NO) [8]. For study the antioxidant level of protection, we have determined the activity of key enzymes of AOD: superoxide dismutase (SOD) and glutathione peroxidase (GPO) [9], as well as the activity of the regulatory enzyme of the pentose phosphate pathway (PPP) of carbohydrates conversion — glucose-6-phosphate dehydrogenase (G6PDH).

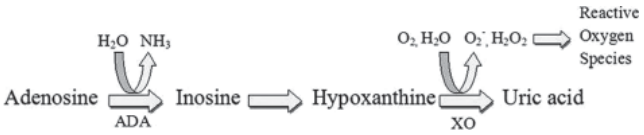

Figure. Schematic representation of the purine nucleotides decomposition

As an indicator of the intensity of the oxidative modification of proteins (OMP), we have determined the content of ketone dinitrophenylhydrazones (KDNPH) and aldehyde dinitrophenylhydrazones (ADNPH) of the neutral and basic character. This is one of the earliest and most stable indicators of lesions of various tissues and cells of the body during the intensification of free-radical oxidation and oxidation of thiol proteins [10]. We determined the values of these parameters in erythrocytes to study the effect of POS and antioxidant system (AOS) in different age groups under normal conditions and in case of CC.

MATERIALS AND METHODS

The determination of mentioned indices was conducted in hemolysate of erythrocytes received by double freezing of washed erythrocytes. 26 patients aged 40–79 years with adenocarcinoma of the rectum III–IV stage

were examined. The control group consisted of 50 conditionally healthy middle-aged and elderly people without pathologies of the gastrointestinal tract or other severe systemic pathology. All examined people were divided into two groups depending on their age: the first age group — 40–59 years, the second — 60–79 years.

The study is conformable to the ethical principles for clinical trials and the provisions of the Helsinki Declaration of the World Medical Association and eliminates infringing the interests of the patient and damage of his health (Commission on Bioethics of Maxim Gorky National Medical University, Donetsk).

ADA activity was determined by changing the optical density of the reaction mixture at a wavelength of 265 nm due to the accumulation of inosine during hydrolytic decomposition of adenosine [11]. Determination of activity of XO is based on the ability of the enzyme to generate superoxide anion radical, the content of which was determined by the rate of recovery of nitro blue tetrazolium to formazan [12]. Endogenous level of NO in the form of nitrite anion (NO_2^-) after enzymatic recovery of nitrates to nitrites was determined by means of standard Griess reaction and designated as NO_x [13]. SOD activity was determined by inhibition of adrenaline autooxidation with adrenochrome formation. GPO activity was determined by the rate of oxidation of reduced glutathione according to the method of Moin [14]. G6PDH activity was determined by increase of NADPH [15]. OMP was evaluated by the method of R.L. Levine modified by E.E. Dubinina [16], OMP products were registered spectrophotometrically at different wavelengths: 356 nm and 430 nm — aliphatic KDNPH of neutral and basic character; 370 nm and 530 nm — aliphatic ADNPH of neutral and basic character. The level of OMP products was given μmol 2,4-dinitrophenylhydrazones generated per 1 mg of erythrocytes protein. Determination of total protein was performed according to the procedure described by Lowry [17]. Identification of all parameters was performed spectrophotometrically (a spectrophotometer Specord-200).

Statistical data processing was performed using the program "Statistica 10.0" Statsoft. Shapiro — Wilk W test was used for checking normality of data distribution. Correlation analysis was performed using Spearman's rank correlation test.

RESULTS AND DISCUSSION

During the study of the control group (conditionally healthy), we discovered a significant decrease with age of enzymatic activities of: ADA, SOD, GPO, NADPH in erythrocytes, whereas the activity of XO with age did not show statistically significant changes. Furthermore, a significant increase in the level of NO_x in the group of elderly people was observed (Table 1).

The obtained data indicate weakening of cellular AOS: decrease in concentration of reduced glutathione (as activity of G6PDH is decreased, which means that formation of NADPH was decreased), increase in the oxidation of thiol groups of enzymes leads to weakening of AOD of erythrocytes and to changing in their metabolism with age. At the same time, increase of OMP products with age was discovered (Table 2), which is another reason for the inhibition of enzymatic link of the AOD system. It contributes to enhancement of oxidative stress [18].

Table 1. Markers of decomposition of purines, POS and AOS in erythrocytes depending on age (M ± m)

Markers	40–59 years (n = 29)	60–79 years (n = 21)
NO_x (mmol/l)	2.95 ± 0.22	3.65 ± 0.24*
ADA (nmol/min·mg)	12.1 ± 0.28	9.7 ± 0.24**
XO (μmol/min·mg)	5.9 ± 0.19	5.75 ± 0.22
G6PDH (nmol/min·mg)	0.42 ± 0.04	0.33 ± 0.03*
SOD (u/mg)	9.3 ± 0.22	7.45 ± 0.21*
GPO (μmol/min·mg)	421.0 ± 7.45	364.0 ± 13.8**

Note: *marker values are reliable when $p < 0.05$; **marker values are reliable when $p < 0.001$

Table 2. Level of OMP products in erythrocytes depending on age (μmol/mg; M ± m)

Age groups (years)	Wavelengths at which the OMP products were recorded			
	356 nm KDNPH (neutral)	370 nm ADNPH (neutral)	430 nm KDNPH (basic)	530 nm ADNPH (basic)
40–59 (n=29)	8.08 ± 0.03	7.30 ± 0.05	5.51 ± 0.06	4.07 ± 0.02
60–79 (n=21)	8.38 ± 0.03*	7.68 ± 0.06*	5.98 ± 0.09*	4.73 ± 0.05*

Note: *marker values are reliable when $p < 0.001$

After the correlation analysis of changes in the parameters of the erythrocytes depending on age, we determined the statistically significant negative correlation of ADA with NO_x (*rho* = 0.33, p = 0.020), i.e., decrease of ADA in erythrocytes and increase of NO acts as a protective mechanism activated in conditions of hypoxia [19]. Also positive correlations of ADA with SOD (*rho* =0.67; p=0.0003), ADA with GPO (*rho* = 0.46; p = 0.001), GPO with G6PDH (*rho* = 0.49; p = 0.001) were discovered, thus pointing to the coordinated operation of enzymatic link of the AOD system. It is of particular interest to note that with age in control group, enzymatic markers of POS and AOD decrease, but non-enzymatic markers of POS significantly increase. Thus, age is one of the determining factors in the correlation of AOD with POS.

In the study of patients with CC in the second age group (with respect to the first one), the decrease of the activities of ADA, GPO and G6PDH was shown, while SOD activity was not significantly changed (Table 3).

Table 3. Markers of decomposition of purines, POS and AOS in erythrocytes in patients with CC depending on age (M ± m)

Markers	40–59 years (n = 10)	60–79 years (n = 16)
NO_x (mcmol/l)	9.2 ± 0.41	13.1 ± 0.81*
ADA (nmol/min·mg)	5.4 ± 0.19	4.3 ± 0.24*
XO (μmol/min·mg)	7.7 ± 0.16	12.3 ± 0.51**
G6PDH (nmol/min·mg)	0.23 ± 0.04	0.15 ± 0.03*
SOD (u/mg)	11.6 ± 0.44	12.1 ± 0.45
GPO (μmol/min·mg)	203.0 ± 8.25	154.0 ± 2.95**

Note: *marker values are reliable when $p < 0.05$; **marker values are reliable when $p < 0.001$

At the same time, in the enzymatic and non-enzymatic link of POS, increase in the activity of XO and NO_x levels with age, as well as increase in OMP level in erythrocytes of patients from the second age group (Table 4) was identified. Therefore, it can be assumed that there is an enhancement of OMP in erythrocytes of older patients with CC, that is accompanied

by changes in the intensity of metabolic processes of the organism and, therefore, further exacerbates the development of oxidative stress. Erythrocytes are highly sensitive to oxidative stress because of contact with high O_2 concentrations. It may result in the autooxidation of hemoglobin, peroxidation of membrane lipids followed by impairment of erythrocyte membrane and limited erythrocyte capability to repair mentioned injuries.

Table 4. Level of OMP products in erythrocytes in patients with CC depending on age (μmol/mg; M ± m)

Age groups (years)	Wavelengths at which the OMP were recorded			
	356 nm KDNPH (neutral)	370 nm ADNPH (neutral)	430 nm KDNPH (basic)	530 nm ADNPH (basic)
40–59 (n=10)	10.6 ± 0.15	9.79 ± 0.09	8.38 ± 0.07	6.63 ± 0.12
60–79 (n=16)	12.1 ± 0.14*	10.6 ± 0.10*	9.49 ± 0.08*	7.52 ± 0.09*

Note: *marker values are reliable when $p < 0.001$

During the comparative analysis of the mentioned parameters in CC patients, there were established statistically significant relationships for: NO_x and XO (*rho* = 0.44; p = 0.032), NO_x and ADA (*rho* = −0.49; p = 0.014) and GPO and XO (*rho* = −0.72; p = 0.0001). The decrease in the activity of ADA in erythrocytes combines with hypoxia is typical for CC. It keeps extracellular adenosine level, thus acting as an adaptive mechanism of cells protection activated in conditions of hypoxia and stimulating increase in production of NO [20]. It is known that the cellular effect of NO depends on the ratio of the concentration of NO and superoxide anion radical in a cell. NO and superoxide anion radical individually are inducers of apoptosis. However, simultaneous increase in the activity of XO and NO levels observed by us in the older age group of patients with CC contributes to the formation of peroxynitrite ($ONOO^-$), thus acting as a protective mechanism of a tumor cell from the cytotoxic action of these metabolites. Furthermore, in comparison with superoxide anion radical $ONOO^-$ is even more powerful oxidizing agent capable of oxidizing both NH- and SH-groups of proteins [21]. This oxidation leads to inactivation of certain enzymes, one of which is SOD, as well as GPO — involved in the deactivation of peroxynitrites with formation of thiol radicals of glutathione (GS). As a result, the last of antioxidants is transformed into prooxidant [22]. This, in its turn, is consistent with the data obtained on decrease in the activity of G6PDH in erythrocytes accumulating NADPH for recovery of glutathione.

We also discovered direct correlation of OMP with NO_x products (*rho* = 0.49; p = 0.041), OMP with XO (*rho* = 0.62; p = 0.008), while activities of enzymatic markers of AOD negatively and significantly correlate with the level of protein oxidation products: GPO with OMP (*rho* = −0.67; p = 0.003), G6PDH with OMP (*rho* = −0.51; p = 0.011). The observed increase in NO_x and OMP with age in conditions of simultaneous decrease of enzymes of antioxidant level of protection is an indication of strengthening of pro-oxidant status of erythrocytes. Therefore, in erythrocytes of older patients with CC, restructuring of the antiradical protection system is observed. This restructuring is closely related to changes in the metabolism of nucleotides. It is known that in healthy cells and cells of benign tumors, oxidizers contribute to increase in cell proliferation, while antioxidants inhibit it, thus acting as a signal that controls cell division [23]. By contrast to this, in cells of malignant tumors, wherein the oxidative stress is characterized by even more expressed manifestation, antioxidants help tumor cells to proliferate and survive by protecting them from apoptosis. But excess of the ROS can also irreversibly damage the regulatory proteins and nucleic acid molecules, thereby contributing to enhancement of metastasing of tumor cells [24].

Thus, determined malfunctions of AOD in erythrocytes have age-related features, which are aggravated in case of tumor pathology, affecting the viability of a tumor cell and its functional full-value, which is more typical in the late stages of the disease. Such malfunctions lead to an imbalance between the POS and AOS, which are closely related to enzymatic changes in the metabolism of carbohydrates and nucleotides, contributing to strengthening of each other on the principle of feedback, which in its turn leads to the development of oxidative stress and as a consequence — to the structural modification, primarily, of biomembranes, enzymes, and nucleotides. Intensity of metabolic processes and pathogenetic restructuring at the cellular level in their turn depend on the severity of these disorders. Therefore, the balance between oxidants and antioxidants is a key issue in the development of cancer, which remains topical up to the present.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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