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STRUCTURAL CHANGES OF SERUM ALBUMIN IN RESPONSE TO OXIDATIVE STRESS CAUSED BY WALKER-256 CARCINOSARCOMA GROWTH

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***Aim*: To assess oxidative stress and structural changes of the serum albumin in rats with transplanted Walker-256 carcinosarcoma (W256) strains with varying sensitivity to doxorubicin (Dox). *Materials and Methods:* The study was performed on female Wistar rats with transplanted W256. On the 9th day after tumor cell transplantation an analysis of peripheral blood, oxidative stress parameters, and structural changes of serum albumin of experimental animals was performed. *Results*: On the 9th day after W256 transplantation a significant increase in the leukocyte counts was observed in the groups of animals with the Dox-resistant and parental (Dox-sensitive) W256 tumors compared with the group of the intact animals: up to $14.24 \pm 1.92 \cdot 10^3/\mu l$ and $9.78 \pm 1.03 \cdot 10^3/\mu l$, *vs* $8.92 \pm 1.04 \cdot 10^3/\mu l$, respectively, due to the increase of granulocyte and monocyte counts. The number of lymphocytes was within the normal range. The level of hemoglobin and the erythrocyte counts were also within normal limits, but hematocrit in both groups of animals with tumors somewhat increased against the background of 1.2-fold elevation of the mean erythrocyte volume. In the group of rats with Dox-resistant W256, there was observed a decrease in the plateletcrit by almost 22% and thrombocyte counts — by 28%. Analysis of oxidative stress indices revealed a significant increase in the level of reactive oxygen species, 2-fold increase of malonic dialdehyde level and the degree of oxidative damage of blood plasma proteins, as well as a decrease in the activity of catalase in hemolysates (by 12–15%) in both groups of tumor-bearing rats. With the use of differential scanning calorimetry, UV and fluorescence spectroscopy we have revealed anomalous conformational changes of albumin caused by tumor development: structural rearrangements in the region of its first drug binding site located in the IIA domain, separation of globular parts of albumin molecule, and partial "opening" in a protein molecular three-domain structure resulting a loss of its thermal resistance. *Conclusion:* The development of transplanted Walker-256 carcinosarcoma, especially its Dox-resistant variant, results in severe metabolic intoxication reflected in alteration of hematological parameters, and indices of oxidative stress, as well as architectonic changes of serum albumin.**

Key Words: **Walker-256 carcinosarcoma, oxidative stress, serum albumin, conformational changes of protein molecule.**

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The overall effect of the growing tumor involves a significant disturbance of metabolic processes in the body. It includes a decrease in the content of some blood cells, shifts in the protein composition of blood plasma, complex changes in the structure and functions of a number of organs, including those that provide detoxification protection of the organism at the conditions of endogenous intoxication [1, 2]. The increase of endogenous intoxication in cancer patients is caused by the products of tumor metabolism and biochemical disorders caused by the tumor enlargement and its mechanical effects. Disturbance of the metabolic processes leads to the development of "metabolic endogenous intoxication".

An important factor in the development of endogenous intoxication is the oxidative stress that occurs as a result of the activation of free radical processes and imbalance of substances with anti- and pro-oxidant properties. It is known that free radical processes are the initiators of further physicochemical changes in the organs, tissues and membranes of cells that can lead to the pathological states and interphase death. Practically at all stages of its formation free radical peroxidation generates a series of products via interaction of free radicals both with each other, and with biological macromolecules. During free radical oxidation, along with reactive oxygen species (ROS), other active compounds (peroxides, epoxides, aldehydes, ketones, alcohols, dialdehydes, etc.) are formed which can covalently interact with certain functional groups of proteins, especially those containing SH-, SCH_3-groups of cysteine, methionine, NH-groups of lysine, etc. [3]. All these agents can cause modification of proteins, including enzymes, the changes of their activity, the destruction of bioantioxidants (vitamins, ubiquinone, steroid hormones, etc.), the changes of the phospholipid composition, the appearance in the hydrophobic part of the oxidation products that initiate the processes of ion transport, the change of the protein conformation and lipid composition, alteration of structural and functional properties of membranes.

An intense increase of ROS and lipid hydroperoxide levels could lead to the rapid destruction of cell structures, but this process is controlled by the complex

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Abbreviations used: CAT – catalase; Dox – doxorubicin; HSA – human serum albumin; LPO – lipid peroxidation; MDA – malonic dialdehyde; MPV – mean platelet volume; OMP – oxidatively modified proteins; PCT – plateletcrit; PDW – platelet distribution width; PLT – platelet count; ROS – reactive oxygen species; TBA – 2-thiobarbituric acid; W256 – Walker-256 carcinosarcoma.

multicomponent enzymatic and nonenzymatic systems of antioxidant defense of the body. They restrain the rate of free radical oxidation processes at a certain physiological level, which is one of the mechanisms of homeostasis.

The harmful effects of free radicals are directed on three types of targets: lipids, nucleic acids and proteins. Affecting the latter, ROS can disrupt their structure and function, causing the oxidation of amino acid residues. By attacking protein molecules, hydroxyl radicals do not remain in solution in the free state, but form the complexes with proteins. Oxidized proteins are mostly functionally inactive, are better exposed to proteolysis, are able to accumulate in different tissues, mediate oxidative DNA damage, and may themselves act as a source of free radicals, depleting the stores of cellular antioxidants.

The central place among blood plasma proteins belongs to human serum albumin (HSA), the blood plasma concentration of which yields up to 45–50 mg/ml. The role of albumin in normal state and in pathological conditions such as cancer is the same: albumin transports fatty acids to the tumor cells, removes metabolic products, as well as mediators produced by the tumor, and transfers them in organs and systems [4–7]. The loading of albumin molecules depends not only on the tumor mass, but also on total amount of HSA in the body: the reduction of HSA in cancer patients is associated with an unfavorable prognosis.

Despite the fact that hydroxyl radicals violate the molecular structure of the albumin molecule, they are tightly bound by it. In this case, the damaged albumin is degraded by proteolytic enzymes, and its loss is quickly compensated by *de novo* synthesis. The potential target for oxidants is its only free SH-group in the position of Cys 34, which could form sulfenic group, causing modification of the protein, change in its activity, polymerization and destruction of amino acid residues [8].

In this study, we have tested the hypothesis that during tumor development ROS can cause significant metabolic shifts and structural changes in the serum albumin molecule in rats with transplanted Walker-256 carcinosarcoma (W256).

MATERIALS AND METHODS

The research was conducted on Wistar female rats 2.5 months old weighting 185.0 ± 13.8 g, bred in an animal facility at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. The work with animals was carried out in accordance with the provisions of the "General Ethical Principles of Animal Experiments", adopted by the First Congress on Bioethics (Kyiv, 2001) and in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (Starburg, 1986). As an experimental tumor model, the W256 strains with different sensitivity to doxorubicin (Dox) were used; the strains were obtained from the National Bank of Cell Lines and Tumor Strains of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine [9].

After tumor transplantion, the rats were randomized by weight and distributed into 3 groups: rats with Dox-sensitive W256 (n = 7), rats with Dox-resistant W256 (n = 7), and intact rats (control group, n = 7).

The animals were slaughtered under a slight etheric anesthesia and blood was collected in tubes with heparin. Blood analysis was performed using automatic blood analyzer Partial Counter PCE-210. Blood plasma was obtained by centrifugation for 15 min at 3000 rpm. A portion of the plasma obtained was frozen and stored at –70 °C.

The total level of ROS generation in blood plasma was determined using a N,N-diethyl paraphenylenediamine probe [10] that in acidic media in the presence of oxygen radicals and Fe^{2+} converts into a colored form with the two-peak absorption spectrum (I_{abs} = 511 and 552 nm).

The activity of catalase (CAT) was determined in hemolysates of blood of experimental rats by the method [11], which is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts.

The level of lipid peroxidation (LPO) was assessed by the content of malonic dialdehyde (MDA) in blood plasma by the method [12], which is based on the ability of MDA to form a stable color trimethine complex with 2-thiobarbituric acid (TBA).

Determination of protein oxidation was carried out by the method [13]. This method is based on the reaction of the interaction of oxidized amino acid residues of proteins with 2,4-dinitrophenylhydrazine and the formation of derivatives of 2,4-dinitrophenylhydrazone.

The study of blood plasma thermal denaturation and albumin fractions isolated by the method [14] was performed using differential adiabatic scanning calorimeter DASM-4 (Pushchino, Russia).

An assessment of the conformational changes in plasma albumin in rats with transplanted tumors compared to intact animals was performed by UV spectroscopy using the DU 70 Spectrophotometer (Beckman, USA) and fluorescence spectroscopy using the Sinergy automatic plate reader (USA) (excitation wavelength λ = 295, emission wavelength λ = 340 nm), as well as NanoDrop ND3330 (NanoDrop, USA) with an excitation wavelength λ = 360 and an emission wavelength λ = 540 nm.

Statistical analysis of the data was performed using the methods of descriptive statistics, correlation analysis, nonlinear regression analysis, Student's *t*-test, Mann — Whitney U-test using Microsoft Excel, Statistic and Microcal Origin software. Data are presented as M ± m, where M is the mean value, m is the standard error of the mean.

RESULTS AND DISCUSSION

Hematologic analysis showed that on the 9th day after transplantation of the Dox-resistant W256 to Wistar rats, a significant increase in leukocyte counts was observed in this group compared with the intact group of animals: 14.24 • 10^3/μl *vs* 8.92 • 10^3/μl due to the increase of monocyte counts from 0.67 • 10^3/μl to 1.28 • 10^3/μl and granulocytes — from 2.25 • 10^3/μl to 7.36 • 10^3/μl. The number of lymphocytes in this case was within the nor-

mal range. In the group of animals with transplanted Dox-sensitive W256, a 9.6% increase in the leukocyte counts was observed, in particular, monocyte counts and granulocyte counts increased by 22.4 and 76.0%, respectively (Table 1).

The level of hemoglobin and the erythrocyte counts were within the normal range and corresponded to those in the group of intact rats, however, the hematocrit level in both groups of rats with tumors was found to be somewhat increased, which is probably due to an increase of the mean corpuscular volume (MCV) from 42.80 ± 0.79 fL in intact animals to 51.09 ± 0.45 fL and 50.82 ± 0.45 fL in rats with Dox-resistant and Dox-sensitive W256. However, this did not affect the MCV-related parameters of erythrocytes: mean cell hemoglobin, the amount of hemoglobin per one erythrocyte and mean corpuscular hemoglobin concentration, the amount of hemoglobin per unit volume, since they were within the normal range (Table 2).

In the group of rats with Dox-resistant W256 a thrombocytopenia was also observed. Platelet count (PLT) in this group decreased by almost 28%, and plateletcrit (PCT) — by 22%, which is probably due to the destruction of platelets. As to the platelet distribution width (PDW), which indicates the heterogeneity of these cells in a certain volume, it did not differ significantly between the groups of tumor-bearing rats and intact animals. Attention is drawn to the index of mean platelet volume (MPV), which presently is considered as a marker of the severity of some pathologies [15] and serves for differentiated diagnosis, as well as for prognosis — its elevation in cancer patients is thought to be associated with a poor prognosis. As can be seen from Table 2, the MPV level in the group of animals with Dox-resistant and Dox-sensitive W256 was higher than in intact controls by 22.9 and 19.8%, respectively ($p < 0.05$). In these groups there was also observed an increase of the MPV/PLT ratio by 71.9 and 37.5% compared to control animals. Elevated MPV/PLT ratio evidences on a poor prognosis as well as some other prognostic indices, in particular, platelet-to-lymphocyte ratio or lymphocyte-to-monocyte ratio [16, 17].

It can be seen in Table 3 that the most negative dynamics of the indices is observed in rats with Dox-resistant W256. This phenomenon is consistent with the data on the production of ROS by peripheral blood neutrophils and monocytes of rats with DOX-resistant W256 [18]. The authors have demonstrated that in this group of animals ROS production by neutrophils increased by 344% ($p < 0.05$) and by monocytes — by 86% ($p < 0.05$) compared to the rats with Dox-sensitive W256 and by 642% ($p < 0.05$) and 263% ($p < 0.05$) respectively compared with the control group of animals. Such an increase in the ROS generation by these blood cells, as well as by the cells of the W256 carcinoma, promotes the development of oxidative stress leading to the enhancement of endogenous intoxication in rats with transplanted tumors.

The data on the intensity of ROS generation in blood plasma of experimental animals are shown in Fig. 1.

The Figure shows that in the groups of tumor-bearing rats the level of ROS generation is much higher than in the group of intact animals. The obtained results indicate a deficiency of activity of protective antioxidant systems of an organism. The antioxidant defense system includes various molecular factors that can prevent the formation of free radicals and initiation of related chain reactions, as well as eliminate already created substances of this

Table 1. The leukogram of intact rats and rats with transplanted W256 (Dox-resistant and Dox-sensitive strains) on the 9th day after tumor transplantation

Blood indices	Intact rats (control group)	Rats with Dox-resistant W256	Rats with Dox-sensitive W256
White blood cell, • 10^3/μl	8.92 ± 1.04	14.24 ± 1.92*$^{\Delta}$	9.78 ± 1.03
Lymphocyte, • 10^3/μl	6.00 ± 0.88	5.60 ± 1.03	5.00 ± 0.92
Monocyte, • 10^3/μl	0.67 ± 0.09	1.28 ± 0.10*$^{\Delta}$	0.82 ± 0.11*
Granulocyte, • 10^3/μl	2.25 ± 0.29	7.36 ± 0.90*$^{\Delta}$	3.96 ± 0.83*

Note: *$p < 0.05$ as compared to intact control group, $^{\Delta}p < 0.05$ as compared to Dox-sensitive W256 group.

Table 2. The hemogram of intact rats and rats with transplanted W256 (Dox-resistant and Dox-sensitive strains) on the 9th day after tumor transplantation

Blood indices	Intact rats	Rats with Dox-resistant W256	Rats with Dox-sensitive W256
Red blood cell, • 10^6/μl	9.51 ± 0.53	9.44 ± 0.22	9.51 ± 0.59
Hemoglobin, g/dl	15.72 ± 0.47	15.82 ± 0.24	15.62 ± 0.74
Hematocrit, %	40.35 ± 0.91	48.48 ± 0.89*	48.34 ± 2.85*
Mean cell volume, fl	42.80 ± 0.79	51.36 ± 0.45*	50.82 ± 0.45*
Mean cell hemoglobin, pg	16.58 ± 0.45	16.72 ± 0.33	16.44 ± 0.29
Mean cell hemoglobin concentration, g/dl	33.00 ± 0.88	32.60 ± 0.56	32.34 ± 0.58
RDW, %	16.08 ± 0.75	15.02 ± 0.29	15.62 ± 0.08
PLT, • 10^3/μl	255.30 ± 65.63	184.60 ± 36.50*	226.20 ± 36.90
PCT, %	0.23 ± 0.01	0.18 ± 0.03*	0.22 ± 0.03
MPV, fl	8.25 ± 0.36	10.14 ± 0.72*	9.88 ± 0.31*
PDW, fl	27.9 ± 0.19	27.40 ± 0.36	27.92 ± 0.26

Note: *$p < 0.05$ as compared to intact control group.

Table 3. Blood indices ratio of experimental animals on the 9th day after tumor transplantation

Ratio	Intact rats	Rats with Dox-resistant W256	Rats with Dox-sensitive W256
MPV/PLT	0.0320	0.055*$^{\Delta}$	0.044*
PLT/lymphocyte	42.55	32.96*$^{\Delta}$	45.24*
Lymphocyte/monocyte	8.95	4.375*$^{\Delta}$	6.09*

Note: *$p < 0.05$ as compared to intact control group; $^{\Delta}p < 0.05$ as compared to Dox-sensitive W256 group.

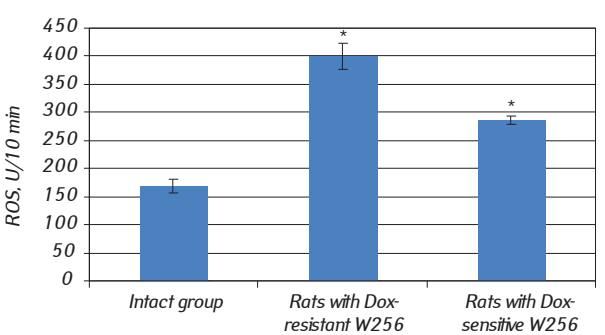

Fig. 1. Intensity of ROS generation in the blood of experimental animals on the 9th day after tumor transplantation compared with intact control. *The difference is significant compared to control (intact) group, $p < 0.05$

nature. An enzyme that provides effective protection of cell structures against degradation by hydrogen peroxide is CAT, the decrease in activity of which can lead to excessive accumulation of ROS in the body.

The results of the study of CAT activity in hemolysates of intact rats and rats with W256 are shown in Fig. 2.

Changes in CAT activity were observed in both experimental groups of animals with tumors compared to intact rats, but they did not differ significantly between themselves. The observed decline in CAT activity in particular, and the antioxidant defense potential in general, lead to an excessive concentration of ROS the negative effect of which manifests itself in the appearance of peroxidation products, which are quite toxic, have high reactivity toward lipids and proteins, and enhance the negative effects of cancer development.

The secondary product of LPO is MDA. The LPO level may be evaluated via determining the MDA content. The data on the content of MDA in blood plasma of rats are presented in Fig. 3. On the 9th day after tumor transplantation, there was registered the 2-fold increase of the MDA level in the both groups of rats with W256 in comparison with the control group. It is also known that in the state of oxidative stress free radicals attack proteins, including proteins of plasma membranes, with the formation of oxidatively modified proteins (OMP). The study of OMP content in blood plasma of rats (see Fig. 3) showed

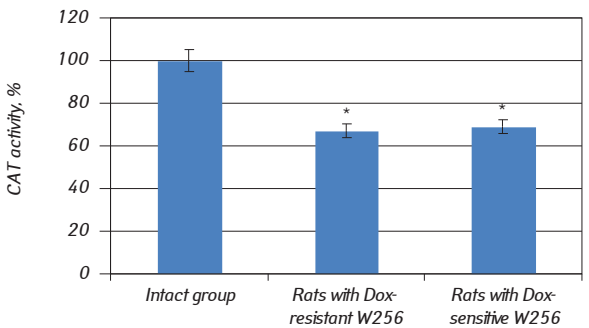

Fig. 2. CAT activity in hemolysates of experimental animals on the 9th day after tumor transplantation compared with intact control. *The difference is significant compared to control (intact) group, $p < 0.05$

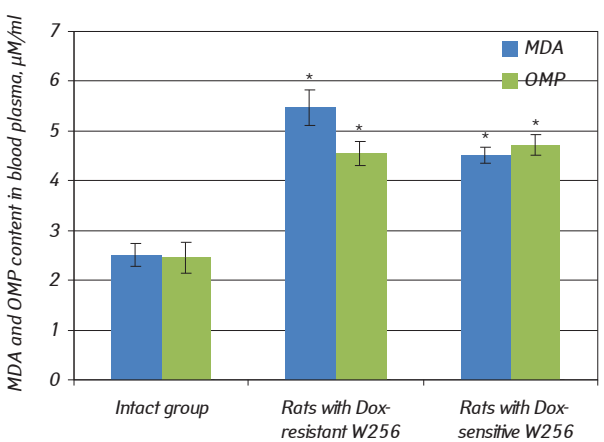

Fig. 3. The content of MDA and OMP in blood plasma of experimental animals on the 9th day after tumor transplantation compared with intact control. *The difference is significant compared to control (intact) group, $p < 0.05$

an increase in the level of oxidatively damaged proteins in blood plasma of rats with W256.

The oxidized forms of some proteins are somewhat resistant to proteolysis and are capable of inhibiting the proteolysis of oxidized forms of other proteins. As a result of oxidative stress, various modifications of proteins can occur, with changed conformation and altered functional activity. The differential scanning microcalorimetry method was used to assess the conformational shifts in the protein spectrum of blood plasma. Fig. 4 demonstrates the melting curves of blood plasma of intact rats and rats with W256 of both strains on the 9th day after transplantation.

Fig. 4 demonstrates that the melting curves of blood plasma of rats from the 2nd and 3rd groups differed notably from those of the control group both by the shape of the endotherms and by the position of the main maximum of thermodenaturation, indicating "pathological" thermodynamic changes in the protein spectrum of the blood plasma of the tumor-bearing rats. From Fig. 4 it is evident that the process of thermodenaturation of blood plasma proteins of rats from different groups occurs in the temperature range from 52 to 82 °C, but the height of the melting endotherm above the baseline of the sample from the 1st group is almost 2 times larger than that of the samples from the 2nd and 3rd groups, and the calculated melting enthalpy (area under the melting curve) was 489.45 ± 47.50, 395.86 ± 35.33 and 380.03 ± 32.27 kJ/(mole • K0) in the 1st, 2nd and 3rd groups, respectively. Reduction of the melting enthalpy in these groups indicates a decrease of the thermodynamic equilibrium in the system, as well as the amount of thermal energy required for the complete denaturation of blood plasma proteins in the rats of groups 2 and 3 as compared with control group, which means partial loss of intermolecular bonds and the thermal stability of blood plasma proteins in tumor carriers, probably associated with changes in their conformation due to the development of the pathological process.

Structural rearrangements in the albumin molecule, the major protein of blood plasma, may occur as a result of its oxidation or overload with hydrophobic ligands of its acceptor centers. To clarify this issue, an extraction of the albumin fraction from the pools of blood plasma of animals from the three groups was carried out using the method of PEG-sedimentation.

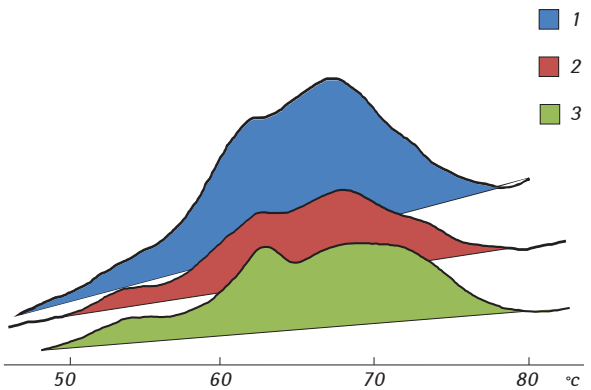

Fig. 4. Melting curves of blood plasma of control animals (1), rats with Dox-resistant W256 (2) and rats with Dox-sensitive W256 (3)

Fig. 5 shows melting curves of albumin preparations isolated from the blood plasma of intact rats and animals with W256 of both strains on the 9th day after tumor transplantation.

Thermograms of albumin fractions isolated from plasma blood of rats with tumors differ from those characteristic for intact animals. First of all, their endotherms lack a thermodenaturation maximum located in the range of temperatures of 62–63 °C. At the same time there is observed an increase of the temperature of the second maximum by 8.0 °C, which indicates significant conformational rearrangements caused by the acceptance and overload of the albumin molecule by ligands of various chemical nature and different origins occurring in the II A domain. The thermograms of albumin melting of rats from both "pathological" groups also show a significantly lower amplitude of elevation over the baseline, indicating the loss of the part of interdomain and inter-subdomain bonds and, consequently, the loss of cooperativity of the process of thermodenaturation. Such anomalous changes directly indicate the presence of essential conformational transitions.

To confirm the presence of conformational changes in the albumin molecule, the absorption spectra of this protein were recorded (Fig. 6). It is known that in far-ultraviolet region, below 240 nm, the absorbance of peptide bonds predominates, peaking near 187 nm. The absorbance is very high but is strongly affected by traces of turbidity or the presence of carboxyl ions. In near-ultraviolet range (240–400 nm) the absorbance spectra of albumins are similar to those of the most of simple proteins with a peak near 280 nm (A_{280}) belonging to the tryptophan residues. The absorbance A_{280} for albumin also includes the contribution from S-S bonds. In the visual spectral range (400–800 nm) solutions of albumin are colorless and absorb no light.

From Fig. 6 one can see an increase of absorbance value of serum albumin of rats with W256 due to separation of globular parts of albumin molecule, and partial "opening" in a protein molecular three-domain structure, wherein tryptophan and some of tyrosine residues initially exposed to some internal hydrophobic surfaces, become accessible to solvent molecules and light source. The observed absorbance spectral changes

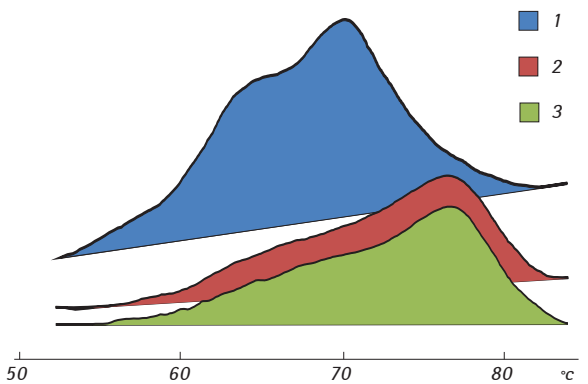

Fig. 5. Melting curves of albumin preparations isolated from plasma blood plasma of control animals (1), rats with Dox-resistant W256 (2) and rats with Dox-sensitive W256 (3)

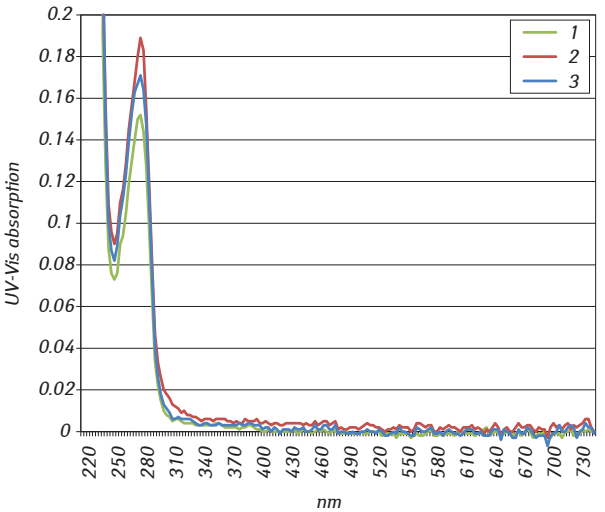

Fig. 6. Absorption spectra of albumin samples isolated from blood plasma of experimental animals: control animals (1), rats with Dox-resistant W256 (2) and rats with Dox-sensitive W256 (3)

of relative spectral absorbance in near-ultraviolet range indicate the occurrence of conformational changes.

The fluorescence spectroscopy is usually used to study the conformation of plasma proteins. Characteristics of the emission of aromatic amino acid residues, such as tryptophan, tyrosine and phenylalanine, can be a convenient tool for studying the change of the conformation of an albumin molecule. Fluorescence is mainly attributed to tryptophane residues in albumin molecule. The more numerous tyrosine residues make a very small contribution, which depends on the wavelength of the absorbing light. When this length ranges from 295 nm to 305 nm, tyrosine is not excited, and there is observed a pure spectrum of tryptophanes fluorescence, in a range close to 345 nm. The serum albumin molecule of rats contains one tryptophan residue in position 214, similar to human albumin.

The data obtained by the fluorescence analysis showed the changes in the tertiary structure of the albumin molecule in rats with W256 (Dox-resistant and Dox-sensitive variants) compared with the group of intact animals (Fig. 7). It could be seen that in the body of tumor carriers, the intensity of the fluorescence of the albumin molecule decreases, which is another evidence on the change of conformation and the presence of allosteric effects affecting the location of the active fluorescence emitter in the protein globulus.

The fluorescence spectra, presented in Fig. 8, are obtained at λ_{ex} = 360 nm and λ_{em} = 540 nm due primarily to the excitation and emission of tyrosines. The Fig. 8 shows more expressed decrease in the albumin fluorescence in rats with Dox-resistant W256 in comparison with albumin of rats from group 3 indicating conformational changes in albumin molecules against the background of pathological changes induced by tumor development.

CONCLUSIONS

The results of this study demonstrated that increase in the ROS generation and deficiency of activity of protective antioxidant systems promotes the development

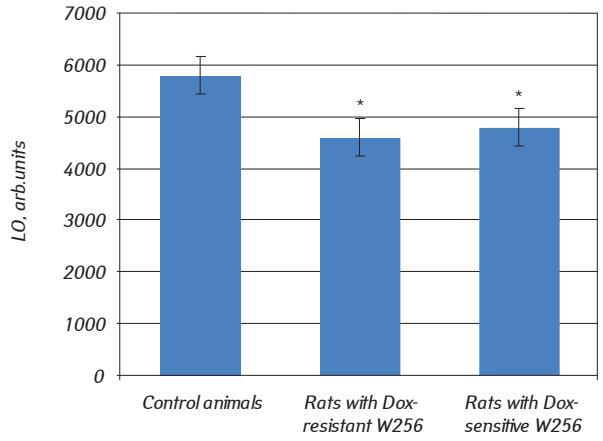

Fig. 7. Intensity of tryptophan fluorescence in albumin samples isolated from blood plasma of rats (λ_{ex} = 295/10 nm, λ_{em} = 340/30 nm). *The difference is significant compared with the intact group, $p < 0.05$

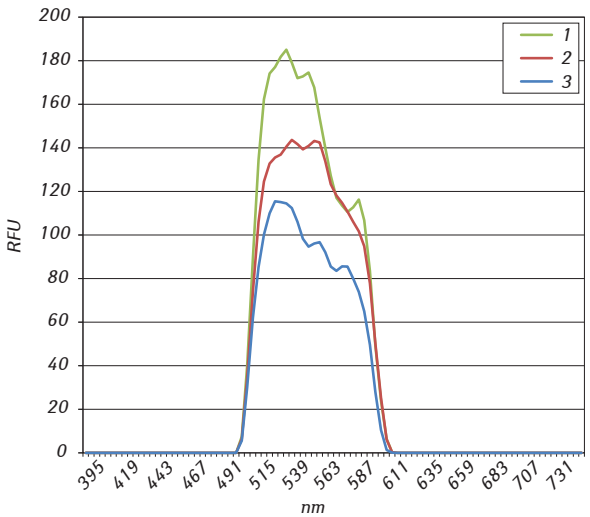

Fig. 8. Fluorescence spectra of albumin samples isolated from blood plasma of experimental animals: control animals (1), rats with Dox-sensitive W256 (2) and rats with Dox-resistant W256 (3), (λ_{ex} = 360 nm, λ_{em} = 540 nm). RFU — relative fluorescence units

of oxidative stress leading to the formation of oxidative-modified serum albumin and enhancement of endogenous intoxication in rats with transplanted W256.

Absorbance and fluorescence spectra of albumin samples isolated from blood plasma of experimental animals demonstrated more pronounced conformational rearrangements of serum albumin molecules in rats with Dox-resistant W256.

The observed changes in hematological parameters, indices of oxidative stress, as well as architectonic changes of serum albumin allow assess the severity of metabolic intoxication caused by the development of transplanted W256.

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