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THE INFLUENCE OF SODIUM DICHLOROACETATE ON THE OXIDATIVE PROCESSES IN SARCOMA 37

L.V. Sorokina[1,2], T.V. Pyatchanina[2], G.V. Didenko[2], A.A. Kaplia[3], S.V. Khyzhnyak[1]
[1]*Taras Shevchenko National University, Kyiv 01030, Ukraine*
[2]*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine*
[3]*O.V. Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine*

***Aim*: to study the activity of antioxidant enzymes and to evaluate an intensity of prooxidant processes in sarcoma 37 (S37) cells during tumor development and under influence of sodium dichloroacetate (SDA). *Methods*: Activity of total superoxide dismutase (SOD), SOD isoforms, catalase (Cat), glutathione peroxidase (GP), and glutathione reductase (GR), as well as content of reduced glutathione (GSH) and lipid peroxidation (LP) secondary byproducts were determined in S37 homogenated tissues of untreated mice and animals treated with SDA at daily dose of 86 mg/kg. *Results*: SDA treatment of S37-bearing mice resulted in the reduced activities of total SOD, SOD isoforms (especially Mn-SOD), Cat, GP and significantly decreased GSH content on the background of LP intensification in tumor tissue. *Conclusion*: The observed changes of oxidative homeostasis in S37-bearing animals treated with SDA could be considered as an element of antitumor action of SDA.**

***Key Words*: sarcoma 37, sodium dichloroacetate, antioxidant enzymes, glutathione-dependent enzymes, lipid peroxidation, tumor growth.**

Tumor growth is characterized by the disturbances in metabolic microenvironment associated with the accumulation of reactive oxygen species (ROS) that provide the modification of intracellular signaling pathways involved in regulation of cell proliferation, differentiation, cellular death (apoptosis, autophagy) and neoangiogenesis, as well as disturbancy in macromolecules and biological membranes [1, 2]. The primary generation of substantial amounts of ROS in cells (including these in malignant neoplasms) is associated with electron transfer by respiratory complexes of inner mitochondrial membrane [2]. The deficiency of respiratory chain components observed in tumor cells creates the conditions for electron leakage outwards the complexes and subsequent free radicals formation [3].

The intensity of prooxidant processes is found to be controlled by antioxidant defense system that comprises enzymatic and non-enzymatic components. The ability of antioxidant system to neutralize ROS is accompanied with involvement of some components of this system within the set of signal transduction pathways associated with cell death and division [4]. The special role is assigned to the antioxidant enzymes localized in mitochondria since they are the primary scavengers of ROS generated during electron flow in respiratory chain and are potentially capable to prevent from the onset of mitochondrial events of apoptosis [1, 4].

The use of substances that cause the death of malignant cells by inducing the metabolic stress mediated by accumulation of the excess amounts of ROS is promising for the cancer therapy [5]. One of the preparations influencing the metabolic processes in mitochondria is sodium dichloroacetate (SDA) that selectively inhibits pyruvate dehydrogenase kinase activated in tumor cells [6]. The possibility of modification of prooxidant-antioxidant homeostasis in tumor cells by the application of SDA *in vitro* is associated with the ability of SDA to affect the formation of mitochondrial ROS [6, 7].

The aim of the study is to investigate the influence of SDA on prooxidant-antioxidant homeostasis of sarcoma 37 (S37) cells in the dynamics of tumor growth *in vivo*.

MATERIALS AND METHODS

Experimental design. 112 male Balb/c mice 2–2.5 months old weighing 20–25 g were used. The experimental procedures with animals were conducted in accordance to the normative rules on bioethics. Sarcoma 37 (S37) cells ($2 \cdot 10^6$ cells per mouse) were transplanted into the thigh muscle. S37 cells that give rise to highly aggressive and invasive tumors after transplantation [8], were cultivated in RPMI-1640 (Sigma, USA) with addition of 10 % fetal bovine serum (Sigma, USA), 2 mM L-glutamine and 40 μg/ml gentamicin at +37°C in humidified atmosphere with 5% CO_2. The viability of cells was determined using trypan blue staining.

The animals with transplanted tumors were divided into 2 groups: 1 — untreated mice (n = 56); 2 — mice that were treated with SDA (intraperitoneal administration at daily dose of 86 mg/kg from the 2nd day after S37 cells transplantation, the total dose of 1548 mg/kg n = 56). The SDA dose was selected on the basis of the previous results and on the data described in [9]. In SDA-treated mice no significant changes of serum blood parameters that characterize the state of liver, kidney, and lipid metabolism were detected that is in accordance with the data described in [7, 10] that showed the absence of any significant toxicity of 30–100 mg/kg SDA per day.

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*Correspondence: E-mail: sorokina_molbiol@mail.ru
Abbreviations used: Cat – catalase; DTNBA – 5,5'-dithiobis-2-nitrobenzoic acid; GP – glutathione peroxidase; GR – glutathione reductase; GSH – reduced glutathione; IMM – inner mitochondrial membrane; LP – lipid peroxidation; NADH – nicotinamide adenine dinucleotide reduced; NADPH – nicotinamide adenine dinucleotide phosphate reduced; ROS – reactive oxygen species; S37 – sarcoma 37; SDA – sodium dichloroacetate; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances.

The S37 growth dynamics was registered by evaluation of tumor size in three dimensions on the 6th, 11th, 14th, 17th, 20th, 24th days after transplantation. The rate of tumor growth was evaluated as tumor volume increase per time interval. The survival of the animals from the both groups was registered. The euthanasia of animals under the light ester anaesthetic was carried out on the 14th, 17th, 20th and 24th day after tumor transplantation.

Isolation of mitochondria and inner mitochondrial membranes. Tumors were washed in physiological saline, the necrosis areas were removed, and tumors were homogenized using "BioMix" device (Germany) in 20 mM Tris-HCl buffer (pH=7.4, +4°C) with 0.32 M sucrose, 2 mM EDTA and 1 mM phenylmethylsulfonyl fluoride. After centrifugation (1500 g, 15 min) the supernatant (S37 homogenate) was further centrifuged at 11000 g for 20 min to isolate mitochondria. The sediment of mitochondria was resuspended in 30 mM Tris-HCl buffer (pH=7.4, +4°C) containing 2 mM EDTA and 0.25 M sucrose.

In order to isolate the inner mitochondrial membranes (IMM) in the form of submitochondrial particles mitochondria were frozen with further thawing and sedimented by centrifugation at 25000–27000 g during 30 min. The purity of the mitochondrial membranes was estimated by marker enzyme assays [11].

The total protein was determined by bromophenol blue assay by Greenberg [12].

Lipid peroxidation (LP) assay. The level of thiobarbituric acid reactive substances (TBARS) in S37 homogenate and in IMM preparations was determined according to [12].

Total catalase and superoxide dismutase activity assays. The activity of catalase (Cat) in S37 homogenates was evaluated using the method [12] based on the measurement of the coloured product of the reaction between ammonium molybdate and hydrogen peroxide. Cat activity was calculated using the calibration curve plotted for catalase purified from human erythrocytes (Sigma, USA). The values of Cat activity are represented in relative units normalized by protein concentration in the sample. One relative unit (r.u.) will decompose 1.0 μmole of H_2O_2 per min at pH 7.0, + 25°C, while the H_2O_2 concentration falls from 10.3 to 9.2 mM, measured by the rate of decrease of absorbance at 240 nm.

The activity of superoxide dismutase (SOD) in S37 homogenates was evaluated using the method [13]. SOD activity was calculated using the calibration curve obtained for SOD preparation from bovine liver (Sigma, USA). The values of SOD activity are represented in relative units normalized by protein concentration in the sample. One relative unit (r.u.) will reduce nitroblue tetrazolium by 50% at pH 7.8, + 25°C in a 3.0 ml reaction volume.

The determination of activity of SOD molecular forms using non-denaturing PAGE. The preparations of tumor homogenates (25 μg of protein) in buffer solution (25 mM Tris-HCl, 5% glycerol, 0.05% bromophenol blue, pH=6.8) were separated in 10% polyacrylamide gel in buffer (25 mM Tris, 50 mM glycine, 0.01% SDS) at 20 mA, 120 V. The protein molecular weight marker (Fermentas, Lithuania) and Cu,Zn-SOD purified from human erythrocytes (Sigma, USA) were used as the standards. After gel incubation during 40 min at room temperature in the dark in reaction mix containing 2 μM nitroblue tetrazolium in 0.036 mM potassium-phosphate buffer (pH=7.8) the activity of SOD was visualized in photochemical reaction using the solution containing 2.8 μM riboflavin and TEMED. Superoxide anions generated during the light-induced riboflavin transformation reduce nitroblue tetrazolium in the sites of SOD activity absence. The activity of SOD molecular forms was detected as the achromatic zones in the violet-stained gel [14]. Using TotalLab v. 2.01 software the relative area of achromatic sites was calculated and expressed in relative units of optical density.

Reduced glutathione and glutathione-dependent enzymes assays. The content of reduced glutathione (GSH) in S37 homogenates was determined in accordance with [15] in the reaction with 5,5'-dithiobis-2-nitrobenzoic acid (DTNBA). The activity of glutathione peroxidase (GP) in S37 homogenates was determined in accordance with procedure based on the evaluation of the oxidized glutathione accumulation in the reaction catalyzed by GP [12]. The activity of glutathione reductase (GR) in S37 homogenates was evaluated by registering the rate of NADPH decrease following the GR-catalyzed reduction of oxidized glutathione [12].

All spectrophotometric measurements were performed in the 96-well transparent polystyrene plates using the multi-mode microplate reader "Synergy HT" (BioTek, USA).

Statistical analysis. Statistical analysis of the data was performed by means of Origin 8.0 for Windows. The significance of statistical differences between two groups was evaluated using the Student's t-test ($P \leqslant 0.05$). The data are presented as mean ± SEMs.

RESULTS

Tumor growth. The kinetics of S37 growth shows three-stage pattern (Fig. 1). The initial lag phase of S37 growth (I stage) lasts up to 11th day after tumor transplantation. Then tumor volume increases by 4 times ($P \leqslant 0.05$) in the period from the 11th to the 17th day (II stage) compared to that at the initial lag period. Since the 18th day up to the 24th day of the experiment (III stage) S37 growth is slower at average by 49% ($P \leqslant 0.05$) in comparison with the preceding II stage. These data allow to distinguish exponential and stationary phases of S37 growth covering the periods from the 11th to the 17th day and from the 18th to the 24th day after tumor transplantation respectively.

The administration of SDA to the tumor-bearing animals at the daily dose of 86 mg/kg resulted in the retardation of S37 growth by 32% ($P \leqslant 0.05$) in the period of II stage and by 53% ($P \leqslant 0.05$) in the period of III stage compared to untreated mice (Fig. 1).

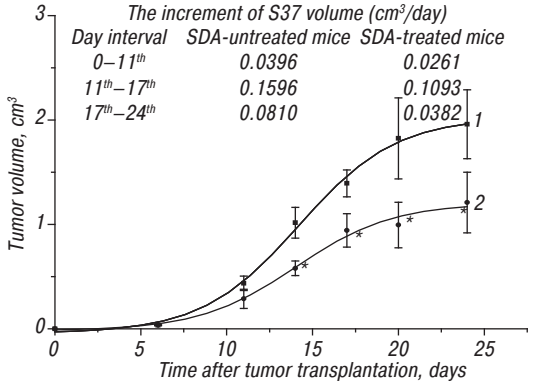

Fig. 1. The kinetics of sarcoma 37 growth in untreated Balb/c mice (1) and in SDA-treated mice (2). * significant differences ($P \leqslant 0.05$).

It should be noted that average life span in the group of SDA-treated animals was increased by 48% ($P \leqslant 0.05$) in relation to untreated mice (34±6 days versus 23±4 days respectively).

The prooxidant processes. During the stage II period of S37 growth the content of endogenous secondary LP products in S37 cells is relatively stable, while at stage III the content of TBARS increases by 37 and 61% respectively, compared to that on the 14th day (Fig. 2, *a*). In S37 cells of SDA-treated mice the enhancement of LP is shown: on the 14th, 17th, 20th and 24th days the TBARS levels in this group were higher at average by 92, 62, 64 and 28% respectively, in comparison to the values for untreated mice (Fig. 2, *a*).

The similar character of TBARS changes was found in tumor IMM preparations (Fig. 2, *b*). The marked elevation of endogenous TBARS level by 142 and 192% was observed on the 20th and the 24th days respectively as compared with that value on the 14th day of tumor growth. In IMM preparations of SDA-treated mice the content of LP products on the 20th and the 24th days was also increased by 32 and 52% respectively compared to the level on the 14th day of S37 growth. The administration of SDA to S37-bearing animals resulted in the augmentation of LP in tumor IMM. On the 14th, 17th, 20th and 24th days the level of TBARS in these IMM increased significantly by 45, 109, 34 and 28% respectively in comparison with IMM from tumors of untreated mice (Fig. 2, *b*).

Fe^{2+}-ascorbate catalyzes nonenzymatic LP in tumor IMM. On the 20th and the 24th days of S37 growth TBARS levels in S37 IMM were higher by 23 and 65 % respectively as compared to the value on the 14th day. On the 24th day of S37 growth in IMM from mice treated with SDA, 30% increase of LP product content was observed as compared with the value on the 14th day (Fig. 2, *c*).

For IMM preparations of S37 of SDA-treated mice the intensity of LP in the presence of Fe^{2+} and ascorbate is higher as compared with that of untreated mice. On the 14th and the 17th days the TBARS content in IMM was increased by 76 and 99% and on the 20th and 24th days — at average by 38–39% in SDA-treated group versus control animals (Fig. 2, *c*).

The enzymes of antioxidant defence. The results of study of the key antioxidant defence enzymes (SOD and Cat) are presented in Table 1. On the 17th, 20th and 24th day of S37 growth Cat activity is lower by 23, 48 and 44% in comparison with the 14th day. The reduction of Cat activity is also inherent for S37 of SDA-treated mice. Cat activity is decreased in these tumors by 46, 51 and 57% on the 17th, 20th and 24th days respectively compared to the values on the 14th day of S37 growth. On the 14th, 17th, 20th and 24th days Cat activity in S37 of SDA-treated mice was decreased by 39, 58, 42 and 53% respectively in comparison with the corresponding values of enzyme activity in tumors of untreated animals.

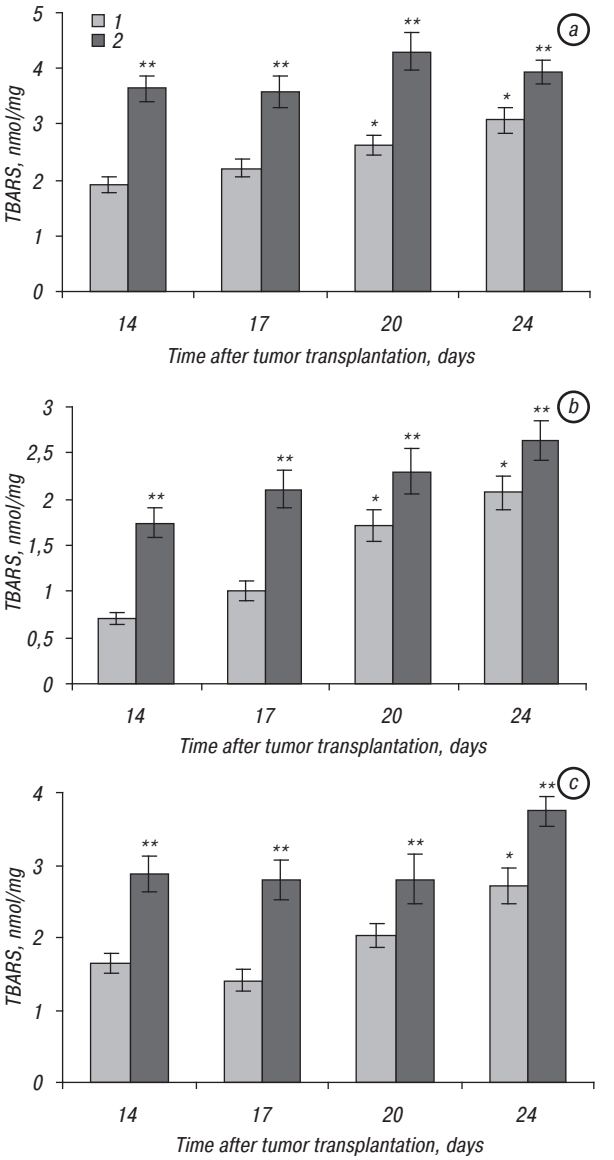

Fig. 2. The content of TBARS in S37 homogenate (*a*), in the IMM of S37 (*b*) and the level of the total TBARS detected in the presence of Fe^{2+} and ascorbate in IMM (*c*) from tumors of untreated (1) and SDA-treated mice (2). Significant differences ($P \leqslant 0.05$): * — *vs*. 14-th day, ** — *vs*. corresponding values for SDA-untreated mice.

On the 17th, 20th and 24th days the activity of total SOD decreased by 20, 24 and 30% respectively in comparison with the 14th day of tumor growth. The S37 growth upon SDA influence was also accompanied with significant decrease of SOD activity on the 17th and 24th days by 20 and 38% respectively compared with that on the 14th day (Table 1). SDA administration also resulted in significant decrease of total SOD activity on the 14th, 17th days by 30% and on the 24th day

by 37% respectively as compared with these values in untreated mice (Table 1).

Table 1. The activity of catalase (Cat) and superoxide dismutase (SOD) in sarcoma 37 during the tumor growth and under influence of SDA

Day of S37 growth	Cat, r.u./mg		SOD, r.u./mg	
	1	2	1	2
14	66.4±5.4	40.3±3.4**	1.55±0.14	1.09±0.10**
17	51.3±4.1*	21.7±2.1*,**	1.24±0.11*	0.87±0.07*,**
20	34.6±3.1*	19.9±1.5*,**	1.18±0.11*	1.01±0.10
24	37.2±3.2*	17.5±1.4*,**	1.08±0.09*	0.78±0.06*,**

Note: 1 – control animals; 2 – SDA-treated animals; r.u. – relative units. Significant differences ($P \leqslant 0.05$): * – *vs*. 14-th day, ** – *vs*. corresponding values for SDA-untreated mice.

The activity of SOD in eukaryotic cells is associated with the existence of enzyme isoforms represented by the Cu,Zn-SOD and Mn-SOD which are characterized by different structure and localization [13]. The dimeric Cu,Zn-SOD (61–64 kDa) and tetrameric Mn-SOD (76–81 kDa) contribute significantly to the total SOD activity in S37 cells. Some part of the observed activity is related to dimeric oe monomeric Mn-SOD with molecular weights of 40–42 kDa and 20–24 kDa respectively and monomeric Cu,Zn-SOD (29–32 kDa) (Table 3).

The activity of tetrameric Mn-SOD underwent the negligible changes during tumor development, but the activity of dimeric Cu,Zn-SOD decreased by 46% on the 24th day of S37 growth as compared with 14th day. The activity of Mn-SOD with lower molecular weight decreased only on the 24th day of S37 growth (at average by 51% compared to the value on the 14th day). At the same time the activity of monomeric Cu,Zn-SOD forms and its separate subunits (16–18 kDa) decreased at average by 44, 39 and 52% ($P \leqslant 0.05$) on the 17th, 20th and 24th days respectively in comparison with the 14th day (Table 2).

SDA treatment resulted in redistribution of activity between different SOD forms resulting in the decrease of the total SOD activity. In the S37 growth kinetics in SDA-treated mice the insignificant changes of tetrameric Mn-SOD activity occured with the exception of the 20th day when the activity of this molecular form is lower by 48% as compared with that on the 14th day. In this group of animals the increase of dimeric Cu,Zn-SOD activity by 29 and 24% was observed on the 17th and 20th day ($P \leqslant 0.05$) respectively as compared with respective value on the 14th day.

The dimeric and monomeric Mn-SOD activities decreased by 24 and 39% on the 17th and 24th days respectively compared with those on the 14th day. The activity of Cu,Zn-SOD with lower molecular weight in S37 of SDA-treated mice is maintained at the relatively stable level except the 20th day when the activity of these forms is higher by 26% as compared with that on the 14th day.

On the 14th day the activity of dimeric Cu,Zn-SOD decreased by 35% in comparison with the activity of this molecular form in untreated mice. On the 17th and the 20th day the activity of dimeric Cu,Zn-SOD idecreased by 16 and 28% and was accompanied with the augmentation of low-molecular form activity by 41 and 63% respectively. On the 24th day in the absence of significant changes for dimeric Cu,Zn-SOD, the activity of monomeric form and subunits of Cu,Zn-SOD increased in tumors of SDA-treated mice by 55% ($P \leqslant 0.05$) in comparison with that for untreated mice (Table 2).

Table 2. The relative activity of SOD isoforms in S37 cells during tumor development

Day of S37 growth		14	17	20	24
Mn-SOD tetramer (76–81 kDa)	1	250.6±20.1	286.4±24.0	277.1±21.1	281.1±23.1
	2	204.1±16.1**	245.1± 20,1	106.2± 23.1*,**	240.2± 20.1
Cu,Zn-SOD dimer (61–64 kDa)	1	364.8±26.1	366.7± 30.1	409.7± 33.0	196.4±20.1*
	2	237.5±20.0**	306.4±22.1*,**	295.4±20.6*,**	189.4±23.1
Mn-SOD isoforms (40–42 kDa, 20–24 kDa)	1	212.2±40.5	225.1±35.1	234.2±31.1	105.0±10.2*
	2	255.3±30.1	190.1±25.4*,**	253.3±25.1	96.0±10.9*
Cu,Zn-SOD isoforms (29–32 kDa, 16–18 kDa)	1	248.1±11.0	140.9±13.1*	153.8±16.1*	120.0±14.1*
	2	198.5±20.1	199.2±28.2**	250.2±20.3*,**	186.4±15.1**

Note: 1 – control animals; 2 – SDA-treated animals. Significant differences ($P \leqslant 0.05$): * – *vs*. 14-th day, ** – *vs*. corresponding values for SDA-untreated mice. Data are represented in the relative units of optical density.

Besides, the consequence of SDA administration was the decrease of total Mn-SOD activity in tumor tissue. In this case the activity of tetrameric Mn-SOD is lower on the 14th and the 20th days of S37 growth by 19 and 62% respectively, and the activity of Mn-SOD forms with lower molecular weight decreased on the 17th day by 16 % as compared with those values in untreated mice.

Glutathione defense system. The reliable changes of reduced glutathione level in tumor cells of untreated mice were not found during observation period. However, in SDA-treated mice GSH content was lower on the 17th, 20th and 24th days of S37 growth by 41, 35 and 40% respectively as compared with that on the 14th day (Table 3). The administration of SDA promoted the exhaustion of GSH pool. On the 14th, 17th, 20th and 24th days of S37 growth the content of GSH decreased by 28, 50, 56 and 55% respectively compared with that in untreated mice.

The activity of glutathione-dependent enzymes (GP and GR) is rather stable during S37 growth except for the 24th day when GR activity decreased by 21% compared with that on the 14th day. However, the administration of SDA to S37-bearing mice resulted in the decrease of GP activity in tumor cells. On the 14th, 17th, 20th and 24th days of S37 growth GP activity was lower by 19, 32, 24 and 27% respectively, as compared with untreated mice (Table 3). Alternatively, on the 14th, 20th and the 24th days of tumor growth GR activity in tumors of SDA-treated mice increased by 22, 40 and 27% respectively compared with that for untreated mice.

Table 3. The content of reduced glutathione (GSH) and glutathione-dependent enzymes in sarcoma 37 during tumor development

Day of S37 growth	GSH, nmol /mg		Glutathione peroxidase, μmol GSSG/ mg·min		Glutathione reductase, μmol NADPH/ mg·min	
	1	2	1	2	1	2
14	48.1±4.1	34.4±3.1**	97.0±7.4	78.1±5.4**	20.5±2.1	25.0±2.0**
17	40.2±3.4	20.3±2.1*,**	101.7±9.1	69.4±5.3**	21.2±2.0	24.5±2.1
20	50.2±4.4	22.2±2.1*,**	99.1±8.4	72.5±6.4**	18.4±1.4	25.8±2.1**
24	46.1±3.1	20.8±1.7*,**	103.1±10.1	78.4±6.3**	16.1±1.2*	20.4±1.7*,**

Note: 1 – SDA-untreated animals; 2 – SDA-treated animals. Significant differences ($P \leqslant 0.05$): * – *vs*. 14-th day, ** – *vs*. corresponding values for SDA-untreated mice.

Hence, sodium dichloroacetate administration results in the depletion of GSH pool in S37 in the phase of active tumor growth. Concurrently GR activity in S37 cells even was increased but GP activity decreased and did not change significantly during tumor growth.

DISCUSSION

As we have shown, S37 growth kinetics includes 3 stages — lag-period, exponential and stationary stages. The inhibition of S37 growth by SDA that is notable from the 14th day corresponds with the data of other authors on the ability of this preparation to cause tumor cell death [6, 9, 16]. Taking into account the involvement of ROS excess in the induction of cell death pathways, it is reasonable to expect that SDA anticancer effect is mediated by influence of SDA on the processes of ROS generation in tumor cells.

The biochemical parameters associated with the prooxidant-antioxidant state were studied in S37 on the 14th, 17th, 20th and 24th days after tumor transplantation. Although the exponential growth of S37 begins on the 11th day the intensity of oxidative processes was analysed on the 14th day due to the experimental conditions (because tumor volume on the 11th day was to small for IMM isolation). Then the parameters were detected on the 17th day when the tumor growth passes from the exponential to stationary phase and on the 20th and 24th days when S37 reached the maximal size.

The higher intensity of LP in S37 cells and in tumor IMM was shown at stationary phase of S37 growth in comparison with exponential phase. The intensification of LP in tumors, particularly in mitochondria, results from the enhancement of metabolic stress, hypoxic microenvironment, insufficiency of mitochondria respiratory chain, inflammatory process in tumour microenvironment [1, 2]. These changes are also evidenced by the activation of Fe^{2+}-ascorbate — dependent LP in IMM of S37 at the same growth stage. The observed LP enhancement resulted in modification of structural and dynamical properties of mitochondrial membranes [17].

SDA-induced activation of oxidative processes in S37 cells, particularly in IMM, is predominantly manifested at the exponential stage of S37 growth as compared with the values for stationary phase. Considering the proposed mechanism of SDA action in acceleration of superoxide anion and hydrogen peroxide generation in tumor cells *in vitro* [6] it could be assumed that SDA-induced accumulation of ROS and products of their transformation may exhibit the toxic properties against cellular biomacromolecules including protein-lipid complexes, nucleic acids, functional groups in the active and regulatory sites of enzymes etc. Apparently, it could result in the subsequent death of tumor cells.

Since activation of oxidative processes in cells can result from the insufficient antioxidant defense systems, we have studied also the functional state of the key antioxidant enzymes. The decrease of total SOD and Cat activities in S37 cells indicated the deficient level of superoxide anion and hydrogen peroxide utilization. The revealed changes are in accordance with the data that showed the relationship between tumor progression and the changes in activity of these enzymes in Lewis lung carcinoma and rat hepatomas 7288, 5123 and 9618A [18, 19].

The activities of total SOD and Cat in S37 cells are supressed upon SDA treatment, but they were not related to tumor growth dynamics and showed no “dose-effect” dependence, so it could be supposed that SOD and Cat activity inhibition is possibly related to modification of enzyme functional groups by active oxygen radicals. Taking into account also the presence of ROS-sensitive sites in the promoter regions of genes encoding SOD and Cat, the following mechanism of SOD and Cat activity suppression could be proposed. High concentrations of ROS block the binding of transcription factors to the sites in gene promoter regions resulting in subsequent depletion of mRNA transcribed from these genes [20].

However, the more detailed analysis of the activity of SOD separate molecular forms indicated its redistribution between different SOD isoforms in tumor growth dynamics in SDA treated mice (in particular, decrease of Cu,Zn-SOD activity (predominantly, the monomeric form) without significant changes of Mn-SOD activity). It might be assumed that changes in the enzyme structure at the posttranslational level due to ROS-driven oxidative modification of functional groups in amino acid residues of the enzyme active site and/or conformational destabilization of the whole enzyme molecule contribute to SOD inhibition in S37 [19].

The activity of dimeric Cu,Zn-SOD slightly decreased in SDA-treated S37, while the activity of low-molecular form increased that could be explained by the possible loss of structural stability and change of conformation properties of enzyme resulting in SOD activity inhibition. It should be noted that the increase of Cu,Zn-SOD monomer activity could be associated with the apparent fragmentation of dimeric Cu,Zn-SOD as a result of conformation destabilization in the presence of free radical oxidants. The negligible decrease of Mn-SOD tetramer activity in SDA-treated S37 could be caused both by oxidative modification due to increase of the intensity of superoxide anion generation in mitochondria and by the inhibition of enzyme by the excess of hydrogen peroxide as a product of enzyme-catalyzed reaction. It was shown [19, 21] that at the early stages of formation of hepatomas and Maloney sarcoma the mitochondrial Mn-SOD activity did not change significantly, but after the appearance of tumor node the sharp decrease of this isoform was observed in the period of maximal rate of tumor growth. Such decrease of Mn-SOD activity enhances the sensitivity of mitochondrial membranes to superoxide anion [1].

The demonstrated decrease of GP activity in SDA-treated S37 indicated the dysfunction in the mechanism of neutralization of lipid hydroperoxides, particularly in biological membranes. Possibly it might be caused by structural rearrangement induced by oxidative modification of the enzyme catalytic/al-

losteric sites or by deficiency of GSH, which content also decreases at these conditions. In spite of some increase of GR activity in S37 upon SDA treatment, one can't exclude the possible utilization of the reactive SH-group of GSH for neutralization of ROS or their derivatives [22].

Thus, the growth of S37 is accompanied by ROS accumulation, particularly in mitochondrial membranes, concurrently with the functional exhaustion of SOD and catalase capacity — superoxide anion and hydrogen peroxide scavenger enzymes. The SDA administration results in the enhancement of lipid peroxidation in S37 cells, including mitochondrial membranes, that is more apparent on the exponential stage of tumor growth. Additional ROS accumulation in S37 cells is caused by suppressed activity of SOD, catalase, and GP. It could be considered as a result of oxidative modification of molecules of these enzymes. In turn, the characteristic redistribution of activity between the different molecular weight SOD isoforms ischaracteristict for S37 cells. The increase of Cu,Zn-SOD monomer activity was shown along with depleted functional ability of enzyme dimer that can be related to the probable fragmentation of dimeric form due to the loss of conformation stability. It should be noted that SDA is capable to enhance lipid peroxidation in mitochondria accompanied with Mn-SOD activity decrease.

Taking into account the available scientific evidences it could be assumed that changes of the intensity of prooxidant processes and functional activity of antioxidant system in sarcoma 37 under SDA influence promote the induction of tumor cell death. Thus, further investigations of the biochemical and molecular mechanisms of anticancer action of SDA seem to be reasonable.

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