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**Biolog. Journal of Armenia, 1 (69), 2017**

# THE STUDY OF THE CHANGES IN ACTIVITY OF SOD IN PATHOPHYSIOLOGY OFGASTRIC CANCER DEPENDING ON THE STAGE OF DISEASE

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Superoxide dismutase (SOD) is an antioxidant enzyme that plays an important role in the defense system of the body. The aim of this study was to investigate the changes in activity of superoxide dismutase (SOD) in the plasma and tumor tissue of patients with gastric cancer depending on the stage (I-IV) of disease. It has been shown that SOD activity in the plasma of gastric cancer patients is significantly reduced compared with SOD activity in the plasma of healthy donors. In the same time tumor tissue SOD activity is increased compared with healthy (histologically examined) control tissue. The results obtained indicated that changes of SOD activity in plasma and tumor tissue of gastric cancer patients depend on the stage of disease.

*Gastric cancer - superoxide dismutase - reactive oxygen species*

Սուպերօքսիդդիսմուտազը (ՍՕԴ) հակաօքսիդանտային ֆերմենտ է, որը կարևոր դեր է խաղում օրգանիզմի պաշտպանողական համակարգում: Այս հետազոտության նպատակն էր ուսումնասիրել ՍՕԴ-ի ակտիվության փոփոխությունը ստամոքսի քաղցկեղով հիվանդների արյան պլազմայում և ուռուցքային հյուսվածքում կախված հիվանդության զարգացման փուլից (I-IV): Ցույց է տրվել, որ ստամոքսի քաղցկեղով հիվանդների արյան պլազմայում ՍՕԴ-ի ակտիվությունը նշանակալիորեն նվազում է առողջ դոնորների արյան պլազմայում ՍՕԴ-ի ակտիվության համեմատ: Միևնույն ժամանակ, ուռուցքային հյուսվածքում ՍՕԴ-ի ակտիվությունն աճում է առողջ (հյուսվածաբանորեն զննված) ստուգիչ հյուսվածքում ՍՕԴ-ի ակտիվության համեմատ: Ստացված տվյալները ցույց են տալիս, որ ստամոքսի քաղցկեղով հիվանդների պլազմայում և ուռուցքային հյուսվածքում ՍՕԴ-ի ակտիվության փոփոխությունը կախված է հիվանդության փուլից:

*Ստամոքսի քաղցկեղ – սուպերօքսիդդիսմուտազ – թթվածնի ակտիվ ձևեր*

Супероксиддисмутаза (СОД) - антиоксидантный фермент, который играет важную роль в системе защиты организма. Целью данной работы было исследование изменений активности супероксиддисмутазы (СОД) в плазме и опухолевой ткани пациентов с раком желудка в

зависимости от стадии (I-IV) заболевания. Было показано, что активность СОД в плазме пациентов с раком желудка значительно снижается по сравнению с активностью СОД в плазме здоровых доноров. В то же время, в опухолевой ткани активность СОД возрастает по сравнению с активностью фермента в здоровой (гистологически исследованной), контрольной ткани. Полученные результаты показали, что изменения активности СОД в плазме и ткани пациентов с раком желудка зависят от стадии заболевания.

*Рак желудка – супероксиддисмутаза – реактивные формы кислорода*

Gastric carcinoma (GC) is one of the most common neoplasms in the world. The pathogenesis of GC is not completely understood. Nutritional, microbial, and genetic factors acting in a multistep and multifactorial process have been proposed [5]. Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer, because reactive oxygen species (ROS) and changes in the cellular redox state activate multiple signaling pathways, participate in the regulation of cell growth and death, and induce the synthesis of proteins regulating cell protection in both nonmalignant and malignant cells [8]. Oxidative stress produced by the imbalance between ROS and biological antioxidant system can damage cellular macromolecules, leading to DNA and protein modification and lipid peroxidation [4].

SOD is a key antioxidant enzyme, scavenging the superoxide radical ($O^{2-}$), which is a precursor molecule for all other reactive oxygen species and their derivatives and can either promote or suppress tumor formation in human gastric mucosa. It has been found out that $Ca^{2+}$/calmodulin (CaM)-dependent protein phosphatase calcineurin (CN), which is key enzyme leading to the activation of the immune system by participating in synthesis of several cytokines via dephosphorylation and activation of NFAT (nuclear factor of activated T cells) transcription factors, is sensitive to oxidative stress and may be modulated by the intracellular redox potential [10]. CN was reported to be a partner of both SOD1 (Cu,Zn-SOD) and mitochondrial SOD2 (Mn-SOD) [11]. Recently, in our laboratory it has been shown, that activity of CN has been changed differently in the pathophysiology of gastric cancer depending on the stage of disease [9]. Considering above mentioned and the controversial literature data concerning the changes in SOD activity in pathophysiology of cancer [6, 13], the aim of this study was to detect the changes of SOD activity in plasma and tumor tissue of patients with gastric adenocarcinoma in all (I-IV) stages of disease.

***Materials and methods.*** Studies were conducted on a group of 30 patients with gastric adenocarcinoma, including males and females, mean age 62 years, who underwent radical gastrectomy for gastric cancer at the National Center of Oncology after V.A. Fanarjyan (Ministry of Healthcare, RA). Patients were not treated by radio- or chemotherapy before surgery. The plasma of healthy donors (n=6) and histologically checked healthy parts of remote tissue were used as a control.

Blood samples (3ml) were collected into sodium citrate (3,2%)-coated vacutainer tubes and plasma was separated by centrifugation at 1500 rpm for 10 min. Tissue samples (1-2g) were homogenized in 5 volumes of 50 mM Tris/HCl buffer (pH 7,5) containing 0,05% Triton-X-100, 0,1 mM EDTA and 1 mM ditiothereitol (DTT) and protease inhibitors. Supernatants were obtained by centrifugation at 10000×g for 60min at $4^0$C. Supernatants and plasma samples were stored at $-70^0$C until determination. Protein content in samples was determined by Bradford assay.

The activity of SOD was measured spectrophotometrically using Agilent Cary 60 UV-VIS spectrophotometer by the method of Sirota [1].

Data were analyzed statistically by one-way ANOVA using Origin 61 software. Statistical significance –$p<0,05$. All data were expressed as mean±SEM.

***Results and discussion***. Results obtained have been demonstrated that in plasma of

gastric cancer patients SOD activity was shown to be decreased in the I, II, III and IV stages by 68.5%, 75.5%, 78.3% and 80.4%, respectively, compared with control (Fig. 1a).

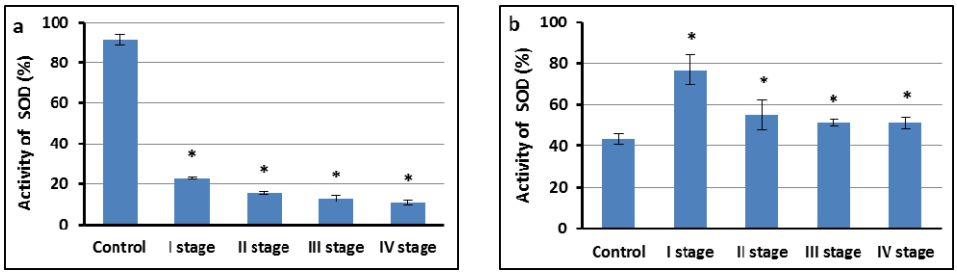


**Fig. 1.** SOD activity in the plasma of healthy donors and gastric cancer patients in different stages of disease (a), and SOD activity in healthy control tissue, as well as in tumor tissue in different stages of disease (b). *$p<0.05$ for the I stage (n=6) compared with control (n=6) and for the II (n=8), III (n=8), and IV (n=8) stages compared with the I stage.

As shown in Fig. 1b, the activity of SOD was considerably higher in tumor tissue compared with the activity in healthy control. SOD activity was shown to be increased in the I, II, III and IV stages by 33.5%, 11.6%, 7.6% and 7.58, respectively, compared with control.

Our data obtained have been indicated that the activity of SOD is changed differently in plasma and tumor tissue of gastric cancer patients among the subsequent stages of GC development. Reduction of plasma SOD activity depending on the stage of GC may be due to an increased endogenous production of ROS in plasma of gastric cancer patients, which is rises from I-IV stage [2]. It is well known that gastrointestinal tract is a key source of ROS, which production has been shown to increase in the gastric mucosa of persons infected with *Helicobacter pylori*, but a large amount of ROS was generated in response to an active inflammatory reaction in the stomach, even among those without *Helicobacter pylori* infection [15]. Despite of the protective barrier provided by the epithelial layer, ingested materials and pathogens can cause inflammation by activating the epithelium, polymorphonuclear neutrophils (PMNs), and macrophages to produce inflammatory cytokines and other mediators that contribute further to oxidative stress and elevated level of ROS [14].

Extensive research during the last quarter century has revealed that reactive oxygen species produced in the body, primarily by the mitochondria, play a major role in various cell-signaling pathways, by activating various transcription factors (e.g., nuclear factor kappa B (NF-κB), activator protein-1, hypoxia-inducible factor-1a, and signal transducer and activator of transcription 3), which have been shown to play an important roles in regulating of constitutive or inductive expression levels of SOD [7].

Paradoxically, ROS also control the expression of various tumor suppressor genes (p53, Rb, and PTEN). Similarly, c-radiation and various chemotherapeutic agents used to treat cancer mediate their effects through the production of ROS, to destroy malignant cells by inducing apoptosis. Interestingly, ROS have also been implicated in the chemopreventive and anti-tumor action of nutraceuticals derived from fruits, vegetables, spices, and other natural products used in traditional medicine [3].

Considering the fact that many human tumors have been shown to express high levels of SOD, which has been associated with aggressive tumor characteristics [12] and our data, we think that up-regulation of SOD activity in gastric carcinoma tissue most likely serves as a protective mechanism against superoxide radicals for cancer cells and targeting SOD may be a promising approach for the selective killing of cancer.

***Acknowledgment.*** We thank to NCO after V.A. Fanarjyan (MOH RA), particularly the Laboratory of Clinical Pathomorphology, for provided oncological samples.

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