DOI 10.4149/BLL_2024_84

Bratisl Med J 2024; 125 (9):
539–543

CLINICAL STUDY

Differential expression of various isoforms of superoxide dismutase in the cells of the human exocrine pancreas

Marko VRZGULA, Jozef MIHALIK, Juraj TESLIK, Ingrid HODOROVA

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia.
marko.vrzgula@upjs.sk

ABSTRACT
OBJECTIVES: Superoxide dismutase (SOD) is an enzyme that plays a crucial role in protecting cells from oxidative damage. Our study aims to address the lack of papers simultaneously analyzing the immunoreactivity of all three distinct isoforms of SOD in human exocrine pancreas cells.
BACKGROUND: Superoxide dismutases (SODs) facilitate the conversion of superoxide radicals into less harmful substances. By neutralizing superoxide radicals, SODs help prevent the formation of highly reactive and destructive species that can adversely affect manifold cellular components.
METHODS: The study analyzed immunoreactivity of SODs in samples of six healthy adult human pancreases, while using the indirect immunohistochemical method under a light microscope.
RESULTS: SOD1 was predominantly found in centroacinar cells and epithelial cells of the duct system while SOD2 was mainly detected in the epithelial cells of interlobular ducts. Both enzymes were prominently present in the basal region of acinar cells near the cell nucleus. The expression of SOD3 was observed to be rare.
CONCLUSION: Understanding the intracellular metabolism of SODs in healthy exocrine pancreas cells serves as a basis for determining the precise role of oxidative damage and SOD signaling in the pathogenesis of various pancreatic diseases, including chronic pancreatitis and pancreatic cancer *(Fig. 6, Ref. 24)*. Text in PDF www.elis.sk
KEY WORDS**:** antioxidants, histology, immunohistochemistry, pancreas, superoxide dismutase.

Introduction

The pancreas is a secondarily retroperitoneal gland with both exocrine and endocrine portions. The exocrine component, characterized by its serous glandular nature, fulfills the function of synthesizing and secreting enzymes into the small intestine. The exocrine pancreas consists of secretory units and a ductal system (1). Structurally, the secretory units, which adopt either a tubuloacinar or acinar morphology, consist of serous acinar cells. The initial segment of the ductal system is comprised of centroacinar cells, which then establish connections with the intercalated duct cells. The intercalated ducts are short and lead into intralobular ducts. The intralobular ducts connect and convey their contents to the interlobular ducts. Finally, the interlobular ducts serve as conduits facilitating the drainage of digestive enzymes into both the main and accessory pancreatic ducts (2).

Superoxide dismutases (SODs), a class of metalloenzymes, are antioxidant enzymes that play a crucial role in scavenging superoxide to prevent oxidative stress. Oxidative stress occurs when the production of reactive oxygen species overwhelms the body's antioxidant defenses, resulting in cellular harm. Superoxide can react with and impair vital cellular components such as proteins, lipids, and DNA, leading to oxidative damage. This damage can disrupt cellular functions and contribute to the development of heterogeneous diseases, including cardiovascular diseases, neurodegenerative disorders, cancer, and aging (3–6). SODs carry out a two-step reaction that converts two molecules of superoxide into one molecule of oxygen and one molecule of hydrogen peroxide through dismutation. By exerting their enzymatic function, SODs regulate the levels of various reactive oxygen species, effectively mitigating the potential adverse effects posed by these molecules. Furthermore, they play a significant role in controlling fundamental aspects of cellular existence through their involvement in signaling pathways (7). Mammals have three types of SODs, each produced by different genes and located in different subcellular regions. Despite these differences, all three types of SODs perform the same reaction (8).

Over fifty years have elapsed since the first identification of principal intracellular SOD, commonly referred to as CuZn-SOD (9). Copper- and zinc-containing SOD1 exists as a homodimer, predominantly found in the cytosol. However, a smaller proportion can be identified in the intermembrane space of mitochondria (10, 11). Additionally, SOD1 has been observed in nuclei, lysosomes, and peroxisomes (12). SOD1 levels vary in response to assorted stimuli including mechanical, chemical, and biological factors such

Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovakia

Address for correspondence: Marko VRZGULA, MD, Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Šrobárova 2, SK-04180 Kosice, Slovakia.
Phone: +421 55 234 3208

Acknowledgments: This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and the Slovak Academy of Sciences VEGA 1/0173/19.

as heat shock, shear stress, UVB and X-irradiation, heavy metals, hydrogen peroxide, ozone, nitric oxide, arachidonic acid, and xenochemicals like β-naphthoflavone and phenobarbital (13). The human SOD1 gene has been identified and mapped to chromosome 21 (14). SOD2, also known as Mn-SOD, is present in a tetrameric form and is initially produced with a leader peptide. This leader peptide directs the manganese-containing enzyme exclusively to the mitochondrial compartments. The SOD2 expression is regulated by cytokines such as interleukin 1, 4, 6, tumor necrosis factor α, and interferon γ (13). In humans, the SOD2 gene is localized on chromosome 6 (15). SOD3, also referred to as EC-SOD, is the most recently recognized SOD isoform. It is an extracellular tetramer containing copper and zinc (16). The expression of SOD3 is controlled by cytokines such as interleukin 4, transforming growth factor β, tumor necrosis factor α, and interferon γ (17). The SOD3 gene was found on chromosome 4 in humans (18).

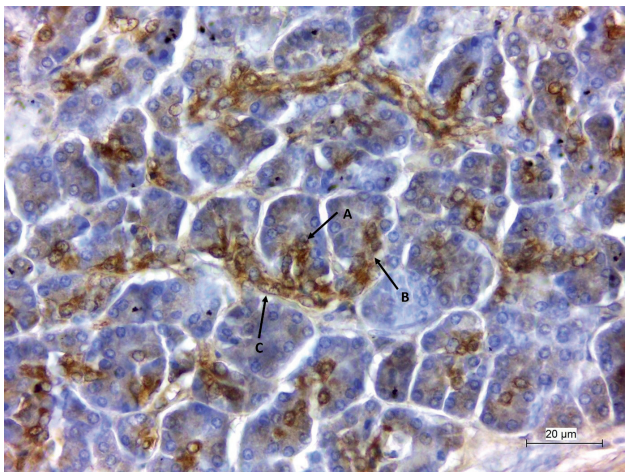

Fig. 1. SOD1 immunoreactivity in the cells of the exocrine pancreas. A-centroacinar cells, B-epithelial cells of intercalated duct, C-epithelial cells of intralobular duct.

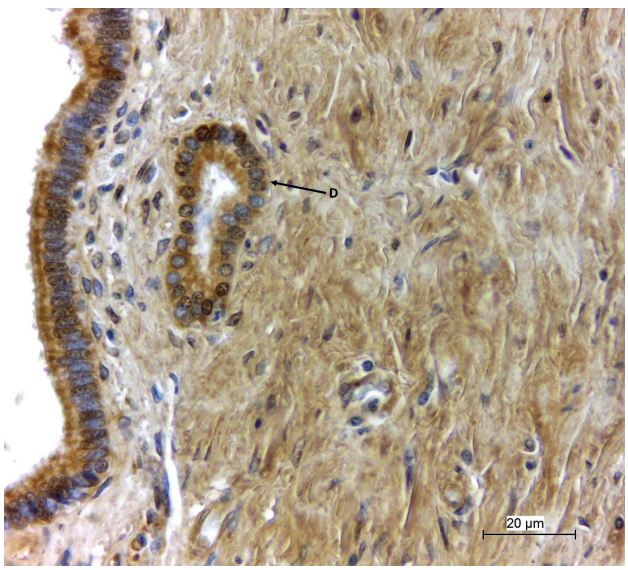

Fig. 2. SOD1 immunoreactivity in the cells of the exocrine pancreas. D-epithelial cells of interlobular duct.

Materials and methods

The study was conducted on six samples of healthy adult human pancreatic tissue fixed in formalin and embedded in paraffin. All pancreatic tissue samples were obtained from the Department of Pathology of L. Pasteur University Hospital in Košice, with the approval of the Hospital Ethics Committee. The processing of the paraffin blocks took place at the Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University in Košice.

Briefly, tissue sections, 3–4 µm in thickness, were prepared using a sledge microtome (HM 400). Deparaffinization, rehydration, and antigen retrieval were carried out using the PT Link system (Dako PT100) and the EnVision™ FLEX Target Retrieval Solution at a pH of 6.1. After a 10-minute incubation in a 3% H_2O_2 solution, the specimens were exposed to primary rabbit polyclonal antibodies. The SOD1-3 antibodies were diluted to a ratio of 1:200 and incubated for 60 minutes.

The tissue sections were then incubated with a biotinylated secondary antibody for 30 minutes. In the next step, the streptavidin-peroxidase solution was applied and allowed to incubate for 30 minutes. The enzymatic conversion of the subsequently added 3,30-diaminobenzidine tetrahydrochloride (DAB) led to a visible reaction product at the antigen site. The specimens were then stained with hematoxylin and embedded in Pertex. Buffer washes using EnVisionTM FLEX Wash Buffer 20x were performed for 5 minutes after each step. As part of each immunohistochemistry run, a negative antibody control (consisting of the buffer without the primary antibody) was included.

Tissue sections were assessed by comparing observations made by two separate evaluators. Digital images were captured using a Leica ICC50 HD camera, connected to a Leica DM500 light microscope.

Results

All three isoforms of SOD were identified in the cells of the exocrine pancreas. Immunopositive cells displayed a brown-to-dark brown cytoplasmic staining, while immunonegative cells had nuclei stained blue with hematoxylin, with their cytoplasm remaining unreactive.

Weak SOD1 positivity was observed, especially in the basal parts of acinar cells. Diffuse and pronounced SOD1 positivity was observed in centroacinar cells, epithelial cells of intercalated ducts, as well as in epithelial cells of intralobular and interlobular ducts (Figs 1,2). SOD2 was predominantly found in the basal region surrounding the cell nucleus in acinar cells. Centroacinar cells and epithelial cells of intercalated and intralobular ducts exhibited weak positivity. Marked positivity of SOD2 was revealed in the epithelial cells of both smaller and larger interlobular ducts (Figs 3, 4). The vast majority of exocrine pancreatic cells were negative for SOD3. Immunohistochemical analysis confirmed that the presence of SOD3 in the studied pancreatic cells was very sporadic,

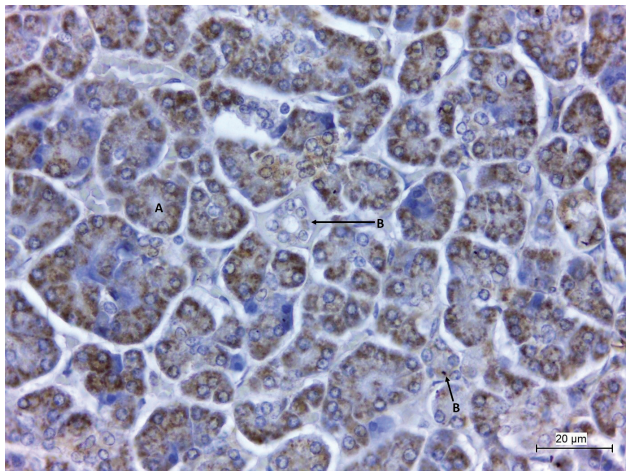

Fig. 3. SOD2 immunoreactivity in the cells of the exocrine pancreas. A-acinar cells, B-epithelial cells of intralobular duct.

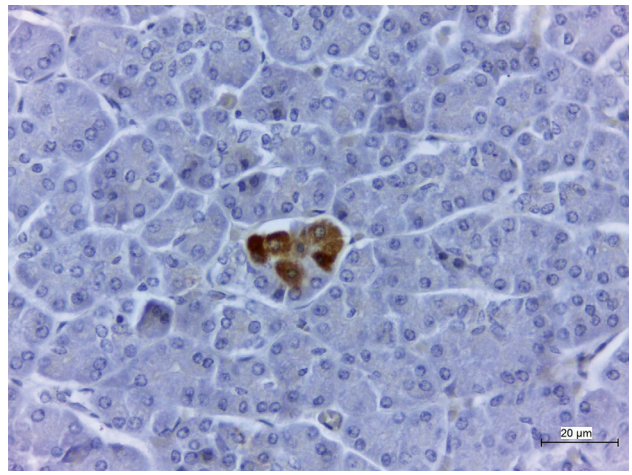

Fig. 5. SOD3 immunoreactivity in the acinar cells of the exocrine pancreas.

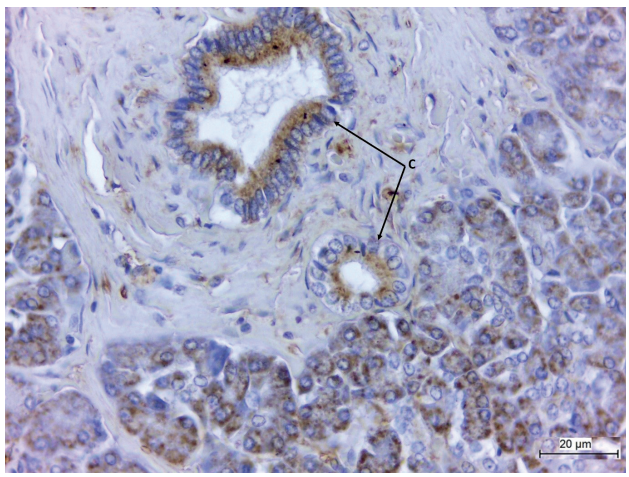

Fig. 4. SOD2 immunoreactivity in the cells of the exocrine pancreas. C-epithelial cells of interlobular ducts.

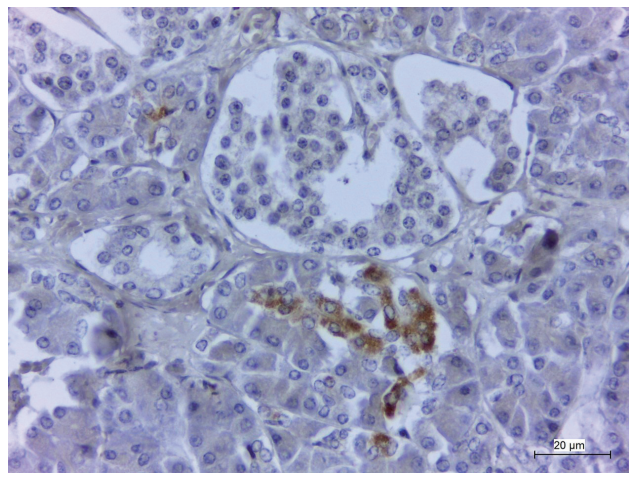

Fig. 6. SOD3 immunoreactivity in the centroacinar cells and epithelial cells of intercalated ducts.

with diffuse distribution in isolated acinar cells, as well as in centroacinar cells and intercalated duct cells (Figs 5, 6). Epithelial cells of intralobular and interlobular ducts in our samples showed no apparent SOD3 positivity.

Discussion

The pancreas functions as a mixed gland, consisting of both exocrine and endocrine sections. The cells of the exocrine pancreas exhibit morphological and functional differences. These cells comprise acinar cells, flat centroacinar cells, and variously shaped epithelial cells of the pancreatic ducts. Acinar cells, the predominant type in the exocrine pancreas, are responsible for producing and releasing digestive enzymes, such as amylase, lipase, and proteases, into the duodenum. They are pyramidal in shape, forming secretory units known as acini. Centroacinar cells, located at the junction between the acini and the pancreatic ducts, serve as a transitional cell population with cytoplasm characterized by a centrally positioned, flattened nucleus. The epithelial cells within the pancreatic ducts demonstrate heterogeneity, varying depending on their location within the duct system. These cells may appear flat, cuboidal, or columnar in shape. While the acinar cells produce a small volume of protein-rich fluid, the cells of the pancreatic ducts secrete a large volume of fluid rich in sodium and bicarbonate. The bicarbonate component helps neutralize the acidic chyme entering the duodenum from the stomach, ensuring an optimal pH environment for the efficient functioning of primary pancreatic enzymes (2).

In the cells of the exocrine pancreas, two primary distribution patterns of SODs were observed. SOD1 and SOD2 exhibited positive staining in acinar cells, particularly in the basal region surrounding the cell nucleus. Serous acinar cells, typical polarized protein-secreting cells, contain eosinophilic secretory granules in their apical cytoplasm. The basal part of acinar cells, housing es-

sential organelles such as the cell nucleus, endoplasmic reticulum, ribosomes, Golgi apparatus, and mitochondria, plays a crucial role in significant protein synthesis. This observation is consistent with the presence of SOD1 and SOD2 in the basal parts of acinar cells. As the most metabolically active region, it is the site of the most significant formation of free radicals and has the greatest need for antioxidant protection. SOD1 and SOD2 were also detected in other cells of the exocrine pancreas. SOD1 showed significant positivity in centroacinar cells, as well as in the epithelial cells lining all pancreatic ducts. Meanwhile, SOD2 was most abundant in the apical parts of epithelial cells of interlobular ducts, with weaker positivity observed in centroacinar and epithelial cells of intercalated and intralobular ducts. Epithelial cells of intercalated and intralobular ducts are actively involved in secreting bicarbonates (HCO3-) to counterbalance acidity, while epithelial cells of interlobular ducts secrete mucin. Given the metabolic activity of these cells, sufficient antioxidant protection is essential, as indicated by the presence of SOD1 and SOD2 within them.

Our study unveiled a distinct distribution pattern of SOD3 in the cells of the exocrine part of the pancreas. The majority of exocrine pancreatic cells in our cohort showed negative staining for SOD3. Given that SOD3 is typically localized in the extracellular matrix and on external cell surfaces, we hypothesize that, influenced by various regulatory factors, cells of the exocrine pancreas produce and secrete this enzyme extracellularly. Consequently, the enzyme was detected only sporadically in some acinar and centroacinar cells, as well as in some epithelial cells of intercalated ducts that were synthetically active at the time of observation. Due to the limited number of healthy pancreas samples in our study, this enzyme might also transiently appear in the epithelial cells of other pancreatic ducts, which subsequently secrete it extracellularly. This hypothesis remains open and requires further verification.

The disrupted balance between agents inducing oxidative stress and defensive mechanisms, such as SODs, is recognized as a detrimental occurrence in numerous diseases, including gastrointestinal disorders like inflammatory disease and cancer (19). In patients with inflammatory bowel disease, elevated levels of SOD in the epithelial cells lining the intestine serve as a protective mechanism against oxidative damage (20). Consequently, levels of SOD in the peripheral blood of patients with inflammatory bowel disease are increased and currently utilized as a biomarker for assessing oxidative stress (21). Oxidative damage has been implicated in multiple aspects of cancer biology, with several studies demonstrating that higher levels of SODs are associated with an increased incidence of invasion and metastasis in various types of cancer, such as gastric and colorectal carcinomas (22, 23). A study by O'Leary et al. (24) demonstrated a significant reduction in SOD mRNA expression in pancreatic ductal adenocarcinoma tissue compared to healthy pancreatic tissue. In our study, we focused exclusively on mapping the occurrence of enzymes in cells of the exocrine portion of the pancreas, omitting consideration of the extracellular stroma or other pancreatic cells. This choice was motivated by the fact that the majority of pancreatic tumors originate from cells of the exocrine pancreas.

Learning points

- The expression of SODs in the cells of the exocrine pancreas demonstrated variability.
- Two primary distribution patterns of SODs have been identified in the cells of the exocrine pancreas: one associated with SOD1 and SOD2, and the other linked to SOD3.
- Comprehension of the intracellular localization and metabolic pathways associated with SODs is a prerequisite for gaining a better understanding of the pathogenesis of various pancreatic diseases.

Conclusion

Our immunohistochemical investigation aimed to address a research gap by concurrently assessing the immunoreactivity of the three distinct isoforms of SOD in exocrine pancreas cells, with the goal of identifying the specific location of each SOD isoform. The findings of this study contribute to the understanding of the intracellular metabolism and regulation of SODs in healthy exocrine pancreas cells, which carries significant implications for modulating the enzymatic antioxidant system for the prevention and treatment of pancreatic diseases.

References

1. Waschke J. Pancreas. 333–337. In: Waschke J, Böckers TM, Paulsen F (Eds). Sobotta Anatomy textbook. Munich: Elsevier GmbH, 2019.

2. Ross MH, Pawlina W (Eds). Histology. Philadelphia: Lippincott Williams & Wilkins, 2011: 647–653.

3. Scioli MG, Storti G, D'Amico F, Rodríguez Guzmán R, Centofanti F, Doldo E et al. Oxidative Stress and New Pathogenetic Mechanisms in Endothelial Dysfunction: Potential Diagnostic Biomarkers and Therapeutic Targets. J Clin Med 2020; 9 (6): 1995: 1–39.

4. Sienes Bailo P, Llorente ME, Calmarza P, Montolio BS, Bravo GA, Pozo GA et al. The role of oxidative stress in neurodegenerative diseases and potential antioxidant therapies. Adv Lab Med 2022; 3 (4): 342–360.

5. Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S et al. Oxidative Stress in Cancer Cell Metabolism. Antioxidants (Basel) 2021; 10 (5): 642: 1–28.

6. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al. Oxidative stress, aging, and diseases. Clin Interv Aging 2018; 26 (13): 757–772.

7. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 2018; 217 (6): 1915–1928.

8. Mondola P, Damiano S, Sasso A, Santillo M. The Cu, Zn Superoxide Dismutase: Not Only a Dismutase Enzyme. Front Physiol 2016; 7: 594: 1–8.

9. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049–6055.

10. Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper,zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci 1992; 89: 10405–10409.

11. Sturtz LA, Diekert K, Jensen LT, Lill R, Culotta VC. A fraction of yeast Cu,Zn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. A physiological role for SOD1 in guarding against mitochondrial oxidative damage. J Biol Chem 2001; 276: 38084–38089.

12. Chang LY, Slot JW, Geuze HJ, Crapo JD. Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes. J Cell Biol 1988; 107: 2169–2179.

13. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol 2002; 33 (3): 337–349.

14. Levanon D, Lieman-Hurwitz J, Dafni N, Wigderson M, Sherman L, Bernstein Y et al. Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu/Zn superoxide dismutase. EMBO J 1985; 4 (1): 77–84.

15. Church SL, Grant JW, Meese EU, Trent JM. Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence *in situ* hybridization and somatic cell hybrid mapping. Genomics 1992; 14: 823–825.

16. Wilkes JG, Alexander MS, Cullen JJ. Superoxide Dismutases in Pancreatic Cancer. Antioxidants (Basel) 2017; 6 (3): 66: 1–9.

17. Strålin P, Marklund SL. Multiple cytokines regulate the expression of extracellular superoxide dismutase in human vascular smooth muscle cells. Atherosclerosis 2000; 151 (2): 433–441.

18. Hendrickson DJ, Fisher JH, Jones C, Ho YS. Regional localization of human extracellular superoxide dismutase gene to 4pter-q21. Genomics 1990; 8: 736–738.

19. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol Rev 2014; 94: 329–354.

20. Kruidenier L, Kuiper I, van Duijn W, Marklund SL, van Hogezand RA, Lamers CB, Verspaget HW. Differential mucosal expression of three superoxide dismutase isoforms in inflammatory bowel disease. J Pathol 2003; 201: 7–16.

21. Rosa AC, Corsi D, Cavi N, Bruni N, Dosio F. Superoxide Dismutase Administration: A Review of Proposed Human Uses. Molecules 2021; 26 (7): 1844: 1–40.

22. Toh Y, Kuninaka S, Oshiro T, Ikeda Y, Nakashima H, Baba H et al. Overexpression of manganese superoxide dismutase mRNA may correlate with aggressiveness in gastric and colorectal adenocarcinomas. Int J Oncol 2000; 17 (1): 107–112.

23. Malafa M, Margenthaler J, Webb B, Neitzel L, Christophersen M. MnSOD expression is increased in metastatic gastric cancer. J Surg Res 2000; 88 (2): 130–134.

24. O'Leary BR, Fath MA, Bellizzi AM, Hrabe JE, Button AM, Allen BG et al. Loss of SOD3 (EcSOD) Expression Promotes an Aggressive Phenotype in Human Pancreatic Ductal Adenocarcinoma. Clin Cancer Res 2015; 21 (7): 1741–1751.

Received January 30, 2024.
Accepted March 14, 2024.