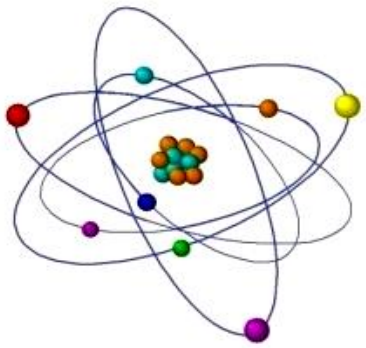
EFFECT OF IONIZING RADIATION ON ATPASES



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ABSTRACT*: Ionizing radiation (IR) has sufficient energy to ionize atoms by detaching electrons from them and promotes the generation of reactive oxygen species (ROS). ROS perform a multitude of signaling functions in different organisms from bacteria to mammalian cells. They were initially considered as a toxic byproducts of aerobic metabolism, but have now been acknowledged as important players in the complex signaling network of cells. Redox signaling is a result of a reversible, covalent modification of specific cysteine thiol residues in active and allosteric sites of proteins, which results in the alteration of protein structure and function. The Na,K-ATPase is activated with an "optimal redox potential range," where the increase of reactive oxygen species (ROS) levels beyond this "optimal range", are responsible for enzyme inhibition. Thus the effect of reactive oxygen species is expressed by biphasic action; stimulation by low doses and inhibition by high doses, which is a manifestation of a hormetic effect. This study was aimed to reveal the effect of IR treatment on the synaptic membrane fractions Na,K-ATPase and Mg-ATPase of the mouse brain. IR (1Gy and 5Gy) treatment of mice results in the alteration of the Na,K-ATPase and Mg-ATPase activity. Na,K-ATPase activity is increased during the initial 3 weeks, after 3 weeks enzyme activity is decreased, while Mg-ATPase activity only increased during the study.*

Key words: Na,K-ATPase, reactive oxygen species, thiol groups, ouabain, ionizing radiation.

INTRODUCTION

As a result of wide use of nuclear energy sources (in particular uranium raw material mining and processing as well as nuclear catastrophes—such as Chernobyl and Fukushima) the level of background ionizing radiation is increased and people are exposed to natural sources of IR from the surrounding environment [1]; IR has sufficient energy to ionize atoms by detaching electrons from them [2] and promotes the generation of ROS. IR is also widely used in research, industry, and medicine [3–6]. Radiation therapy is an accepted therapeutic procedure in oncology. The effects of radiation therapy are achieved, by the production of free radicals. IR can present a health hazard when proper measures against excessive exposure are not taken. Therefore, the adequate determination of its deleterious effects on organisms is one of the essential problems of modern public health. Accidents at nuclear power plants cause severe body irradiation, which can lead to skin burns or acute radiation syndrome, whereas low doses of IR increase the risk of long-term effects, i.e., cancer [1]. IR causes cell damage to various organ systems and tissues [3, 6-8]. Radiation-induced increase in free radicals results in lipid peroxidation, leading to structural and functional damage to cellular membranes and membrane-associated proteins, including ion channels and transporters, which can contribute to the death and survival of irradiated cells [9].

IR-induced alterations may include a decrease in enzyme activity [10, 11] and a decrease in the membrane abundance of transporters. The damage to membrane organization and function is an initial step in cell death. During radiotherapy, formation of ROS in membranes would result in the damage of membrane bound transporters and enzymes. One of these enzymes can be Na,K-ATPase and Mg-ATPase. These ATPases have a crucial role in the maintenance of ion balance. The Na^+,K^+ pump is an electrogenic transmembrane ATPase, a member of the P-type ATPases family, that was discovered in 1957 by Skou [12]. Na,K-ATPase exchanges 3 sodium to 2 potassium, for the free energy of ATP hydrolysis. Na^+ and K^+ move against the concentration gradient, and the pump maintains the gradient of a higher concentration of sodium extracellularly and a higher level of potassium intracellularly. This concentration gradient is crucial for many processes within the cell. Na,K-ATPase also has an essential role in maintaining the resting membrane potential, regulating cell volume, and cell signal transduction. The physiological consequences of Na,K-ATPase regulation are important and play a crucial role in the adaptation of organisms to different conditions [13]. Many stimuli induce the specific modification of Na,K-ATPase and a change in Na,K-ATPase activity [14-17]. One of which is a group of chemically reactive molecules derived from the partial reduction of molecular oxygen, known as reactive oxygen species. These reactive species include oxygen-containing free radicals, such as superoxide anion radical ($O_2^{\bullet-}$) and hydroxyl radical (HO•), as well as hydrogen peroxide (H_2O_2). Due to their high reactivity, ROS are toxic to cells and damage a variety of macromolecules, including nucleic acids, proteins, and lipids [18]. Aside from their toxicity, ROS are important signaling molecules [19; 20]. The redox sensitivity of Na,K-ATPase was first shown in electric eels under a study of the effects of H_2O_2 [21]. This dual effect of p H_2O_2 was further demonstrated in other animal species, tissues, and with other species of ROS, such as hypochlorous anion, hydroxyl radicals, and superoxide. Despite the known effects of ROS on Na,K-ATPase, there is still a lack of information concerning the molecular mechanism of Na,K-ATPase alteration in the tissues after irradiation. Investigations of the enzyme properties in such condition enable us to understand the processes involved in the maintenance of ion homeostasis in tissues after IR.

MATRRIAL AND METHODS

Experiments were performed on age- and weight-matched mice of both genders (10 weeks). The animals were subjected to one-time total-body IR procedure. One group (n=10) received 1Gy radiation, another group 5Gy radiation (n=8). Third group served as control, animals which do not received irradiation. After IR the mice were housed into different cages and observed.

Brain synaptic membrane fractions obtained from different group mice served as the investigation material. The synaptic membrane fraction was obtained using differential centrifugation; at 1.2-0.8 M concentration gradients of sucrose, according to de Robertis [22] recommendations.

Na,K-ATPase activity was measured as an ouabain sensitive part of total ATPase activity. Mg-ATPase was considered as the ouabain insensitive part. The total ATPase incubation medium contained 140 mM NaCl, 5 mM KCl, and 50 mM Tris-HCl buffer at pH 7.7. Mg-ATPase was determined by adding 1 mM ouabain into the medium, consisting of 145 mM KCl and 50 mM Tris-HCl buffer at pH 7.7. Protein concentration for the activity assay was 0.0044mg/ml. The samples were incubated at 37 ^oC for 15 minutes. ATPase activity was calculated according to the amount of inorganic phosphorus (Pi) (per mg protein and per hour), resulting from enzyme-induced ATP hydrolysis. The inorganic phosphorus levels were evaluated calorimetrically [23-

24]. Amount of protein was determined by the procedure of Lowry [25] using bovine serum albumin as a standard.

The experiments were subjected to statistical analysis. The following data is presented as an arithmetic means with a standard error. Differences between two groups were assessed using an unpaired two-tailed Student's t-test. A P value of $p < 0.05$ was considered statistically significant.

The rats experienced no suffering prior to decapitation. All experiments were approved by the animal care and use committee at the Ivane Beritashvili Center of Experimental Biomedicine

RESULTS

ROS are one of the redox signaling molecules produced by IR. The targets for this signaling pathway are cysteine (Cys) residues of proteins, where various thiol modifications also take place. To investigate the effect of IR on the Na,K-ATPase and Mg-ATPase, we have studied changes in enzyme activity after IR exposure. From the Fig.1, it is clear that IR at 1Gy activates the Na,K-ATPase system during 3weeks. Enzyme reaches maximal velocity at the 3 week mark and gradually decreases after 3 weeks.

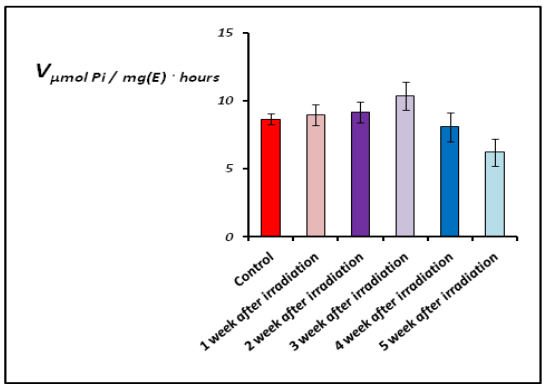

Fig.1 Dependence of synaptic membrane Na,K-ATPase activity on IR (1Gy). The reaction medium was [MgATP]=1.69mM; [Mg_f^{2+}]=[ATP_f]=0.31mM.(*P* < 0.01).

In the case of high dosage of IR (5Gy), activity of Na,K-ATPase is again increased after 1week (Fig.2).

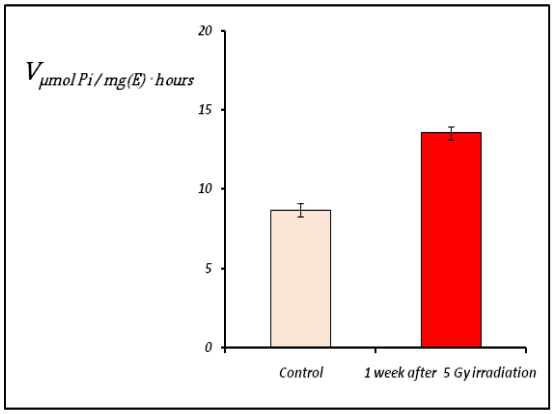

Fig.2 Dependence of synaptic membrane Na,K-ATPase activity on IR (5 Gy). The reaction medium was [MgATP]=1.69mM; [Mg_f^{2+}]=[ATP_f]=0.31mM.(*P* < 0.01).

Activity of Na,K-ATPase after 1 week is higher in the case of 5Gy IR compared with 1Gy IR (Fig. 3)

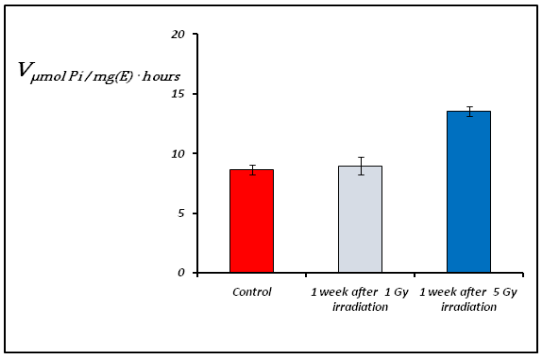

Fig.3 Dependence of synaptic membrane Na,K-ATPase activity on IR. The reaction medium was [MgATP]=1.69mM; [Mg_f^{2+}]=[ATP_f]=0.31mM.($P < 0.01$).

Different results were obtained in the case of Mg-ATPase study (Fig.4). After 1week Mg-ATPase activity was decreased, while after 2 weeks It's activity is increasing gradually during 5 weeks and does not undergo inhibition. (Fig.4).

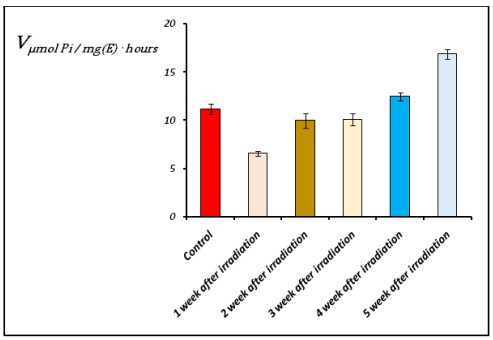

Fig.4 Dependence of synaptic membrane Mg-ATPase activity on IR (1Gy). The reaction medium was [MgATP]=1.69mM; [Mg_f^{2+}]=[ATP_f]=0.31mM.($P < 0.01$).

In the case of a high dose of IR (5Gy) the same change of Mg-ATPase activity is manifested as was obtained during 1Gy IR (Fig.5). Mg-ATPase activity is decreased after 1week and is increased during the second and third weeks after IR exposure.

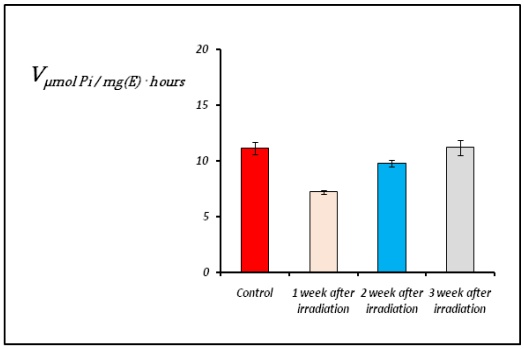

Fig.5 Dependence of synaptic membrane Mg-ATPase activity on IR (5Gy). The reaction medium was [MgATP]=1.69mM; [Mg_f^{2+}]=[ATP_f]=0.31mM.($P < 0.01$).

DISCUSSION

It is well established, that exposing cells to IR causes immediate free radical formation leading to ROS generation, and thus, results in cell damage. In healthy cells, ROS are serving multiple signaling functions, and the body's antioxidant systems, including thiols, control their activity by neutralizing the excessive free radicals. IR-induced increase in free radicals are known to initiate lipid peroxidation, which leads to cell membrane damage [26]. Changes in membrane lipid composition and oxidative state could have dramatic consequences for Na,K-ATPase enzymatic activity [27]. Accordingly, an increase in the concentration of lipid peroxidation products was previously correlated with inhibition of the Na,K-ATPase [27]. Elevation of endogenous ouabain upon IR treatment was also detected [28].

Ouabain is known to specifically inhibit the Na,K-ATPase, it has also been shown to activate this enzyme [29-31] at concentrations comparable to the endogenous ouabain level. Endogenous ouabain is known as a hormone synthesized in the zona glomerulosa cells of the adrenal cortex from cholesterol [28]. IR activates sympathetic nervous system and stress–hormone axis [28], which, in its in turn, regulate endogenous ouabain synthesis and release. Notably, in contrast to the suppressive action of free radicals on the Na,K-ATPase, free radicals have been suggested to be an important component of the oxidative stress amplification loop [28]. The binding of ouabain to the Na,K-ATPase has been suggested to activate multiple signaling pathways that can increase the generation of mitochondrial ROS, which, in turn, can further modify the Na,K-ATPase and potentiate this signaling [28]. Redox signaling is a result of reversible, covalent modification of specific cysteine thiol residues in active and allosteric sites of proteins, which results in the alteration of protein structure and function. ROS oxidizes cysteine residues in proteins to produce disulfides (R-S-S-R) via the formation of a sulfenic acid (R-SOH) intermediate by a biologically reversible reaction [32]. Disulfides can be formed differently; between adjacent cysteines within a protein, known as intra-protein disulfide, between two proteins (interprotein disulfide), or as a mixed-disulfide formed between a protein thiol and glutathione, termed S-glutathionylation. Thioredoxins (Trx) or glutaredoxins (Grx) protein are responsible for reversible reduction of the protein, resulting in a reversible signal transduction mechanism. Further oxidation of protein thiols by H_2O_2 to higher oxidation states form sulfinic (R-SO_2H) and sulfonic (R-SO_3H) acids, within an irreversible reaction [32].

The sodium potassium pump is polymer, formed by three different subunits. The largest is the 100–113 kDa catalytic α subunit, responsible for catalytic activity and ion transport; the others are regulatory subunits – the 55 kDa β subunit and the tissue-specific regulatory proteins of 7–11 kDa members of the FXYD family [33]. Each Na,K-ATPase subunit type contains cysteine residues. The only reduced thiol within the beta subunit resides at the edge of the membrane surface, immersing into and out of the membrane during the pumping cycle [33]. The FXYD subunit has two thiols [34]. The catalytic α subunit has binding sites for ATP and for ions, forms a transport pore, and 23–24 thiols are presented, depending on the isoform [34]. These thiols of different subunits are potential targets of regulatory thiol modifications. We can speculate that ROS at low concentrations reacts with thiolate (S-) from Cys, forming a sulfenate (SO^-) that can result in the formation of disulfide bonds (SS) along with other thiols and activation of the enzyme. Increasing ROS concentration oxidizes the sulfenate to sulfinate (SO_2^-), and finely to sulfonate (SO_3^-), resulting in the irreversible oxidation of Na,K-ATPase and ROS-mediated toxicity. So, from our experimental results we can say, that at the beginning stage, after 1Gy and 5Gy treatment IR stimulate ROS formation and increases Ouabain level. Reversible modification of regulatory thiols of all subunits of Na,K-ATPase by ROS and its signaling pathway stimulates Na,K-ATPase and increase its activity (Fig.1; Fig.2); Further elevated ouabain binds to the Na,K-ATPase activates pathways that can increase the generation of mitochondrial ROS, by Na,K-

ATPase oxidant amplification loop, which, in turn, irreversibly modify enzyme thiols and cause Na,K-ATPase inhibition (Fig.1). In case of Mg-ATPase, elevated ROS levels are not sufficient to irreversibly modify enzyme, and there is only activation of the enzyme system (Fig4; Fig.5).

From our experimental results, we can ascertain that IR treatment of mice results in the alteration of the Na,K-ATPase and Mg-ATPase activity. Na,K-ATPase activity is increased during the initial 3 weeks after IR, after 3 weeks enzyme activity is decreased, while Mg-ATPase activity is increased during observation.

REFERENCES

[1] Kennedy, A.R. (2014). Biological effects of space radiation and development of effective countermeasures. *Life Sci. Space Res*., 1, 10–43.
[2] Ryan, J.L.(2012). Ionizing radiation: The good, the bad, and the ugly. *J. Investig. Dermatol.*,132, 985–993.
[3] Zhou, T.; Lu, L.; Wu, S.; Zuo, L. (2017). Effects of Ionizing Irradiation on Mouse Diaphragmatic Skeletal Muscle. *Front. Physiol.*, 8, 506.
[4] Biau, J.; Chautard, E.; Verrelle, P.; Dutreix, M. (2019). Altering DNA Repair to Improve Radiation Therapy: Specific and Multiple Pathway Targeting. *Front. Oncol.*, 9, 1009.
[5] Kaloˇcayová, B.; Kovaˇciˇcová, I.; Radošinská, J.; Tóthová, L.; Jagmaševiˇc-Mézešová, L.; Fülöp, M.; Slezák, J.; Babál, P.; Janega, P.; Vrbjar, N. (2019). Alteration of renal Na,K-ATPase in rats following the mediastinal γ-irradiation. *Physiol. Rep.*, 7, 13969.
[6] Wang, L.Y.; Yang, P.Y.; Chen, Y.J.; Wu, H.D.; Huang, Y.H.; Hsieh, C.H.(2019). Inspiratory muscle training attenuates irradiation-induced diaphragm dysfunction. Am. *J. Transl. Res.*, 11, 5599–5610.
[7] Russell, J.A.; Connor, N.P. (2014). Effects of age and radiation treatment on function of extrinsic tongue muscles. *Radiat. Oncol.*, 9, 1–15.
[8] D'Souza, D.; Roubos, S.; Larkin, J.; Lloyd, J.; Emmons, R.; Chen, H.; De Lisio, M. (2019). The Late Effects of Radiation Therapy on Skeletal Muscle Morphology and Progenitor Cell Content are Influenced by Diet-Induced Obesity and Exercise Training in Male Mice. *Sci. Rep.*, 9, 6691.
[9] Huber, S.M.; Butz, L.; Stegen, B.; Klumpp, D.; Braun, N.; Ruth, P.; Eckert, F. (2013). Ionizing radiation, ion transports, and radioresistance of cancer cells. *Front. Physiol.*, 4, 212.
[10] Matsuda, H.; Giblin, F.J.; Reddy, V.N. (1982). The effect of X-irradiation on Na-K-ATPase and cation distribution in rabbit lens. *Investig. Ophthalmol. Vis. Sci.*, 22, 180–185.
[11] Hitschke, K.; Buhler, R.; Apell, H.J.; Stark, G. (1994). Inactivation of the Na,K-ATPase by radiation-induced free radicals. *Evidence for a radical-chain mechanism. FEBS Lett.*, 353, 297–300.
[12] Skou J. (1957). Influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochemistry Biophysics Acta*, 23, 394-401. https://doi.org/10.1016/0006-3002(57)90343-8.
[13] Blanco G. & Mercer R.W. (1998). Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am. J. Physiol*. 275, F633–F650 doi: 10.1152/ajprenal.1998.275.5.F633.
[14] Chkadua G., Nozadze E., Tsakadze L., Shioshvili L., Leladze M., Arutinova N., Dzneladze S., Javakhishvili M., & Kupradze S. (2022). Some Kinetic Features of Na,K-ATPase and Sensitivity to Noradrenalione. *Cell Biochemistry and Biophysics*, V. 80, issue 1, 23-29. doi:10.1007/s12013-021-01032-6.
[15] Hernandez J. (1992). Na,K-ATPase regulation by neurotransmitters. *Neurochemistry Int.*, 20 (1), 1-10. doi: 10.1016/0197-0186(92)90119-c.
[16] Vague P., Coste T.C., Jannot M.F., Raccah D., & Tsimaratos M. (2004). C-peptide, Na,K-ATPase and diabetes. *Exp. Diabesity Research*, 5, 37-50. doi: 10.1080/15438600490424514.
[17] Vizi A. & Seregi A. (1982). Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxadation. *Biochemisry Pharmacology*, 31(13), 2231-2236. doi: 10.1016/0006-2952(82)90106-x.
[18] Stadtman E.R. (1992). Protein oxidation and aging. *Science*. 257,1220–1224.doi: 10.1126/science.1355616.
[19] Finkel T. (2011). Signal transduction by reactive oxygen species. *J. Cell Biol.*, 194, 7–15

doi: 10.1083/jcb.201102095.
[20] Rhee S.G., Kang S.W., Jeong W., Chang T.S., Yang K.S.,& Woo H.A. (2005). Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr. Opin, Cell Biol.*, 17,183–189 doi: 10.1016/j.ceb.2005.02.004.
[21] Liu J., Kennedy D.J, Yan Y., & Shapiro J.I. (2012). Reactive Oxygen Species Modulation of Na/K-ATPase Regulates Fibrosis and Renal Proximal Tubular Sodium Handling. *International Journal of Nephrology 3:* ID381320 doi:10.1155/2012/381320
[22] De Robertis E. (1969). Structural components of the synaptic region. *Structural Neurochemistry,* 2,365-380 doi: 10.1007/978-1-4615-7157-5_15.
[23] Fiske G.H. &Subbarow Y. (1925). The colorimetric determination of phosphorus. *Biological Chemistry*, 66, 375-400. https://doi.org/10.1016/S0021-9258(18)84756-1.
[24] Kazanov A. & Maslova M. (1984). The investigation of activation of Na,K-ATPase in the red blood cells of mammals. *Journal of Evol. Biochemistry and Physiology*, 16 (5), 81-87.
[25] Lowry O., Rosenbrough N., & Randall R. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193 (1), 265-275. doi:10.1016/S0021-9258(19)52451-6.
[26] Purohit, S.C.; Bisby, R.H.; Cundall, R.B. (1980). Chemical damage in gamma-irradiated human erythrocyte membranes. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.*, 38, 159–166.
[27] Cornelius, F.; Habeck, M.; Kanai, R.; Toyoshima, C.; Karlish, S.J. General and specific lipid-protein interactions in Na,K-ATPase. *Biochim. Biophys. Acta*, 2015, 1848, 1729–1743.
[28] Violetta V. Kravtsova, Arina A. Fedorova, Maria V. Tishkova, Alexandra A. Livanova, Oleg V. Vetrovoy, Alexander G. Markov, Vladimir V. Matchkov and Igor I. Krivoi (2022). Chronic Ouabain Prevents Radiation-Induced Reduction in the α2 Na,K-ATPase Function in the Rat Diaphragm Muscle; *Int. J. Mol. Sci.*, 23, 10921
[29] Holthouser, K.A., Mandal, A., Merchant, M.L., Schelling, J.R., Delamere, N.A., Valdes, R.R. Jr., Tyagi, S.C., Lederer, E.D., Khundmiri, S.J.(2010). Ouabain stimulates Na-K-ATPase through a sodium/hydrogen exchanger-1 (NHE-1)-dependent mechanism in human kidney proximal tubule cells. Am. *J. Physiol. Ren. Physiol.*, 299, F77–F90.
[30] Ketchem, C.J., Conner, C.D., Murray, R.D., Du Plessis, M., Lederer, E.D., Wilkey, D., Merchant, M., Khundmiri, S.J. (2016). Low dose ouabain stimulates Na-K ATPase α1 subunit association with angiotensin II type 1 receptor in renal proximal tubule cells. *Biochim. Biophys. Acta*, 1863, 2624–2636.
[31] Klimanova, E.A., Tverskoi, A.M., Koltsova, S.V., Sidorenko, S.V., Lopina, O.D., Tremblay, J.m, Hamet, P., Kapilevich, L.V., Orlov, S.N. (2017). Time- and dose-dependent actions of cardiotonic steroids on transcriptome and intracellular content of Na+ and K+: A comparative analysis. *Sci. Rep.*, 7, 45403.
[32] Cremers C.M. & JakobU. (2013). Oxidant sensing by reversible disulfide bond formation. *Biol.Chem.,* 288 (37), 26489-26496. doi: 10.1074/jbc.R113.462929.
[33] Toyoshima C., Kanai R., & Cornelius F. (2011). First crystal structures of Na+,K+-ATPase: new light on the oldest ion pump. *Structure*, 19, 1732–1738. doi:10.1016/j.str.2011.10.016.
[34] Bogdanova A., Petrushanko I., Boldyrev A., & Gassmann M. (2006). Oxygen- and Redox-Induced Regulation of the Na/K ATPase. *Curr. Enzyme Inhibit.*, 2, 37–59. doi:10.2174/157340806775473490.