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Hormesis Effect of Radon in Rats of the Krushinsky-Molodkina Line

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Abstract

According to this research, the use of radon inhalation in experimental animals, particularly in genetically determined rats with epileptic seizures, altered all parameters of the epileptic seizure development picture, namely the hidden period and the first and second wild jog duration after the audiogenic signal. On the third day, no response to the audiogenic signal was observed at all, and there was not even a single episode of tonic-clonic seizures. All what was mentioned suggests that radon inhalation can be used to treat epilepsy. This study is the first precedent of attempting R-Ho through inhalation for treatment of epileptic seizures in animal models with further translation to clinical study in humans through pilot phase II study. More profound and scientifically systematized approach is needed to determine the uniqueness of Tskhaltubo water springs, investigating the mechanisms of radon effects on the excitatory and inhibitory functioning of CNS, and the use of further clinical studies to establish its effectiveness on humans.

Keywords: Radon, Epileptic rats, Oxidative stress, Sulfhydryl groups, Na⁺ / K⁺ ATPase

Introduction

It is known that the basis of the complex functional organization of the central nervous system (CNS) is the coordinated interaction of the main processes of nervous action - excitation and inhibition. In the CNS, these processes are carried out through neurotransmitters (mediators). Neurotransmitters affect the corresponding receptor areas of the neuronal membrane and cause the transmission of information from neuron to neuron or effector cell . The mechanisms of these two processes are similar because in both cases, mediators cause a change in ion permeability in the membrane.

However, the change in the potential of the subsynaptic membrane in the case of the excitation process is due to changes in the permeability of sodium ions, while the inhibition process is associated with changes in the permeability of ions of potassium, chlorine, or both (Bangasser et al., 2014; DiNuzzo et al., 2014).

Coordinated interaction of excitatory and inhibitory potentials contributes to the equilibrium of these processes. Various changes in pre- and postsynaptic mechanisms can lead to an imbalance between excitation and inhibition and switching to an abnormal mode of action. Thus, this occurs in cases of some neurological and mental illnesses, and the most obvious example of this is epilepsy (Bangasser et al., 2014; Bazzigaluppi et al., 2017).

According to most researchers, in the process of excitation-inhibition, the inhibitory function of the CNS is performed by GABA-amino acid (GABA), glycine and taurine, while the excitatory function is associated with glutamine and asparagine acids. The main energy source of neuronal activity is glucose. As a result of anaerobic and aerobic conversions of glucose, ATP is synthesized, which is the energy basis of active processes in the nervous system. Glycolysis takes place in the cytoplasm, resulting in 2 molecules of adenosine triphosphate (ATP) and pyruvate instead of 1 molecule of glucose (DiNuzzo et al., 2014; Martin et al., 2016).

Changes in glucose metabolism deficiency in the action of Na^+ / K^+ ATPase, as mentioned above, are associated with neuronal hyperactivity. This is one of the leading mechanisms of reducing the concentration of extracellular K and what was accumulated after convulsive activity (Isaksen et al., 2016).

Observations on numerous clinical and experimental studies have revealed the pathogenetic role of oxidative stress. Epileptic seizures occur against the background of oxidative stress and hypermetabolic state, which was also mentioned in this article (Yuta Shibamoto et al., 2018; Zharkinbekova et al., 2019). Hence, all this was done in the context of oxygen-generated activity. Seizures increase blood flow to the brain, thus increasing the need for oxygen and glucose, causing the blood vessels to dilate and blood pressure to rise, and leading to the formation of nitric oxide and adenosine. Despite such a sudden increase in oxygen and glucose during the convulsive processes, the energy expenditure is so great that this energy balance in the brain is depleted very quickly and the hypermetabolic state in the period between seizures changes into a hypometabolic state. This is followed by impaired mitochondrial function and oxidative stress (Hashemian et al., 2017; Lomidze et al., 2012).

What is the mechanism that explains the link between impaired glucose metabolism (often glucose hypometabolism) and epileptogenesis? Two hypotheses have been formulated on this issue: The first mechanism is based on the neurotransmitter, especially the gamma-amino acid (GABA)

change in the system. The functioning of neurotransmitter systems requires quite a lot of energy. For GABA-ergic neurons, the gamma-aminobutyric acid uptake mechanism plays an important role in GABA resynthesis, whereas glutamatergic neurons are primarily dependent on glutamine as a precursor of glutamate derived from astrocytes. Accumulation of glutamate causes damage to glutamate receptors, activation of Na^+ and Ca^{++} channels, accumulation of Na^+ and Ca^{++} ions inside the cell, and accumulation of K^+ ions in the cell fluid (Pandey et al., 2012).

An important role in the theory of epileptogenesis is also given to the membrane theory, i.e., the trigger factor. The cause of neuronal epilepsy is a structural change in the neuronal membrane including the synapses, and this leads to the inactivation of ion pumps and abnormal hyperactivity of ion channels. Changes in glucose metabolism deficiency in the action of Na^+ / K^+ ATPase, as mentioned above, is associated with neuronal hyperactivity. This is one of the leading mechanisms to reduce the concentration of extracellular K^+ accumulated after convulsive activity. Low Na^+ / K^+ ATPase activity is associated with the development of epileptic seizures. In addition, Na^+ / K^+ ATPase activity reduce within a few minutes after transient focal ischemia in rat cortex and hippocampus and in an experimental model of brain trauma. Altered ion homeostasis may also partially explain the interaction between convulsive activity and hypoglycemia (Bazzigaluppi et al., 2017). Due to the above, changes in glucose metabolism deficiency during oxidative stress and disruption of ongoing processes involving Na^+ / K^+ ATPase may be considered as one of the reasons for the development of epilepsy (Silva et al., 2011).

Nitric oxide (NO) plays an important role in the peripheral microcirculation and activation of central hemodynamic processes, as well as in the regulation of ion exchange of Na^+ / K^+ and Ca^+ . Activation of nitric oxide (NO) and the formation of hydrogen peroxidase (H_2O_2) occur during various reactions, one of which is exposure to small doses of radiation (hormesis) when the presence of H_2O_2 stimulates the production of excess NO in microphages. As a result of this, NO can be considered in the context of an autocrine homeostatic modulator (Ryan et al., 2011).

Small doses of radiation have long been used in medicine to treat various diseases. Nonetheless, the focus of this study is on the change in excitation-retention reactions under radioactive gas radon in the natural environment during epilepsy (Yuta Shibamoto et al., 2018).

This study was based on research on the effects of radon inhalation on aggressive rats. It was found that after 5 minutes of inhalation daily for 7 days, aggressive rats lost aggressiveness and became non-aggressive and their noradrenaline level increased (Nikolashvili et al., 2006). It is important to note that these changes occurred 1 month after the end of inhalation procedures and the effect lasted for 6 months. However, this suggests that the process is not

carried out by direct chemical reactions, but by the activation of ongoing processes and by strengthening the body's immune system.

Radon is a radioactive gaseous element that has mainly alpha radiation and is actively used in cases of age-induced brain disorders and hypertension (Lomidze et al., 2012). Another well-known positive effect of radon baths is the intensification of the action of such perfusing agents that increases the level of adrenaline in the blood plasma (Jobe, 1973; Rossa, 2000; Yuta Shibamoto, 2018).

Based on all the above, the main goal of the study is to investigate the effect of Tskaltubo radon-containing water inhalation on the development / course of convulsive reactions in experimental animal models of epilepsy in particular, the change in the antioxidant processes in the body, and the change in the concentration of Na/K ATP (Krushinsky, 1970; Marshalick, 199; Nikolashvili, 2021). Genetically seizure-determined Krushinsky-Molodkina (KM) rats were placed in the audiogenic stimulation chamber (Morandi, 2015). The chamber represented the Plexiglas box of 60x60x60cm³ with standard call on upper part of the box. A high pitch sound stimulus was presented to rats (110dB, during 60s) in response to which they developed seizure reactions (Pandey, 2012). Motor components of seizure activity were estimated by a slightly modified scale (Krushinsky, 1970): 0-fear reaction; 1-facial muscle clonus; 2-head tremble, jaw myoclonus; 3-wild running reaction, forepaw myoclonus; 4-myoclonus of fore and hindpaws, fall on the side; and 5-clonus of the four paws, skeletal muscle rigidity, ataxia, asphyxia (Marshalick, 1991).

Material and Methods

Animals: For our experiment, 24 months Krushinsky-Molodkina (KM) line male rats was used. They are predisposed to audiogenic epilepsy (seizures in response to a strong sound). Rapid (5 to 7 seconds) development of clonic-tonic seizures and the development of postictal catalepsy are characteristic of KM rats (Hashemian, 2017)

Epileptic Seizures: Genetically seizure-determined Krushinsky-Molodkina (KM) rats were placed in the audiogenic stimulation chamber (Krushinsky, 1970). The chamber represented the Plexiglas box of 60 × 60 × 60 cm³ with standard call on upper part of the box. A high pitch sound stimulus was presented to rats (110 dB, during 60 s) in response to which they developed seizure reactions. Motor components of seizure activity were estimated by a slightly modified scale: 0-fear reaction; 1-facial muscle clonus; 2-head tremble, jaw myoclonus; 3-wild running reaction, forepaw myoclonus; 4-myoclonus of fore- and hindpaws, fall on the side; 5-clonus of the four paws, skeletal muscle rigidity, ataxia,

asphyxia (Ross, 2000]. Long-term (15 minutes) exposure of KM rats to the action of sound, according to a special scheme with alternating 10 s periods of strong and weak sound, causes cerebral circulation disorders in them, which was externally manifested in the form of paresis and paralysis of the limbs. On the 5th day assessment of epileptic seizure - with trigger - sound in BK rats was performed.

Radon Measurement: In Tskaltubo spa center, where natural mineral water is used, Radon`s radioactivity was measured in water. The radioactivity of Radon was 37 becquerel (bk) in 1m^3 (37 bk/m^3).

Radon Inhalations Procedure: Ten (10) experimental animals (KM rats) was placed in Tskaltubo mineral water spa`s sauna (experimental group). Mineral water temperature was 36°C , Humidity 90%. For the control group, 10 KM rats was placed in another spa center`s sauna with 36°C mineral water (without radon) delivered via inhalation. Humidity in this spa center`s experimental room was 90%. None (experimental and control group of the rats) of the animals took a bath, they were just in two different saunas and living in the same conditions. Inhalation was taken through the nose for 10 minutes, once a day for 3, 5 and 10 days, in conditions of high humidity (about 90%). After each procedure of inhalation, the rats were placed in a vivarium and given food and water.

Laboratory Examination

To study the physiological changes caused by the inhalation of Tskaltubo water on oxidative level, which prevents the development of brain disorders associated with peroxidation reactions, the free radicals concentrations (d-ROM) - reactive oxygen metabolites in the blood plasma of rats was measured using a photometric test. Also, the concentration of hydroperoxides (ROOH) in the brain tissue was also measured, which gives us a pro-oxidant status of the tissue. Hydroperoxides, also called Reactive Oxygen Metabolites (ROM), are formed during an oxidative attack when Reactive Oxygen Species (ROS) react with various organic substrates (e.g., carbohydrates, lipids, amino acids, proteins, nucleotides, etc.).

To assess the antioxidant capacity of plasma, the PAT (Antioxidant Concentration Test) was used by measuring ferric reducing ability and evaluating the effectiveness of antioxidants. In addition, the OSI (Oxidative Stress Index) and the OBRI (Oxidation Balance Status) was determined.

All named measurements were provided by means of Photometric Analytical System FRAS5 (H&D, Parma, Italy) (Nikolashvili, 2021).

Cysteine thiols and their oxidized disulfide counterparts are carefully balanced to maintain redox homeostasis in various cellular compartments, protect organisms from oxidative and xenobiotic stressors, and partake actively in redox-regulatory and signaling processes. In this review, the role of protein thiols as scavengers of hydrogen peroxide in antioxidant enzymes

was discussed. Thiol peroxidases is used to exemplify how protein and non-protein thiols group contribute to redox signaling, provide an overview over the diverse set of low molecular weight thiol-based redox systems found in biology, and illustrate how thiol-based redox systems have evolved not only to protect against but to take full advantage of a world full of molecular oxygen (Raimondo, 2015; Sedlak, 1968) (Determination by ELISA kit).

Data Processing Algorithms and Statistical Procedures: Modern multidimensional and multiparametric statistical methods such as dispersion analysis (MANOVA) were used for the complex processing-generalization of the obtained experimental material STATISTICA-7.

Results

Epileptic seizure

On the 5th and 10th day after inhalation of low doses ($37\ 3700\ \text{Bq} / \text{m}^3$) (Table 1 and Figure 1), the latent period of inhalation of radon before attacks and the pauses between attacks significantly increased ($p < 0.05$) in the group of radon irradiation when compared to the control group. The latency period before inhalation of radon in rats with epilepsy was 11 ± 1.1 seconds, and on the 5th and 10th day after inhalation of Tskhaltubo randomized water, the latency period increased to 16 ± 1.4 and 18 ± 1.3 seconds, respectively. The duration of the first and second jumps after the trigger was reduced in the group of rats receiving radon inhalation ($p < 0.05$). In all groups, the wild run began immediately after receiving a sound signal (call), but the duration of the first wild run decreased from 10 ± 1.1 seconds (control group) to 3 ± 0.1 seconds on the fifth day after inhalation of radon. It even decreased longer by tenths the day after inhalation with the radon reduced to 1.5 ± 0.1 seconds.

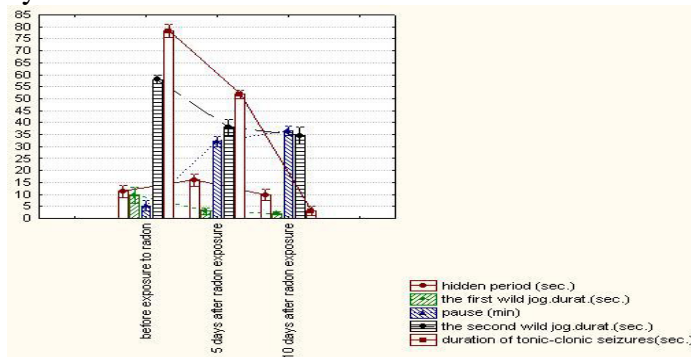


Figure 1. Epileptic seizure

After receiving a sound signal, the duration of the second run decreased from 58 ± 1.7 seconds to 39 ± 1.1 seconds on the 5th day after inhalation and to 35 ± 1.1 seconds on the 10th day after inhalation. The duration of generalized audiogenic tonic-clonic seizures in the radon inhalation group on day 5 after

inhalation was 52 ± 1.3 seconds. As can be seen from Table 1 below, on the day 10 after inhalation – 2 ± 0.1 seconds, the effect of radon causes an increase in the latency period for 5 and 10 days after inhalation.

Table 1. The effect of Radon inhalation on the epileptic seizure in rats

	Hidden period (sec.)	The first wild jogging duration (sec.)	Pause (min.)	The duration of the second wild jogging (sec.)	Duration of tonic-clonic seizures (sec.)
Before exposure to radon ($p < 0.05$)	11 ± 1.1	10 ± 1.1	5 ± 0.3	58 ± 1.7	78 ± 1.9
5 days after radon exposure ($p < 0.05$)	16 ± 1.4	3 ± 0.1	32 ± 2.2	39 ± 1.1	52 ± 1.3
10 days after radon exposure ($p < 0.05$)	18 ± 1.3	1.5 ± 0.1	36 ± 2.2	35 ± 1.1	2 ± 0.1

From the data presented in Table 2, the study of dROM in genetically epileptic Molotkin's Krushinsky rats before exposure to radon showed very high oxidation status (521 ± 3.67). On the 5th day after exposure to radon, the level of oxidative stress was 381 ± 2.95 , which is statistically significant ($p < 0.05$). The hematological concentration of antioxidants (PAT plasma with antioxidant fragments) was at the edge before being taken to Tskaltubo in epileptic rats (2091 ± 3.95). As a result of inhalation of radon, it became 2763 ± 8.5 which is statistically reliable ($p < 0.05$). OBRI (Oxidation Balance Risk Index): if it was before radon inhalation (1.95 ± 0.3), it is dangerously high for the organism. After inalation, it was recorded as high but this data was not dangerous for the organism (1.6 ± 0.2).

Table 2. Oxidative stress in epileptic rats

Epileptic rat	D-ROMs FAST Ucurr.	PAT	OBRI	OSI REDOX
Control	521 ± 3.67 Free radicals, very high	2763 ± 5.85 Antioxidants There is a deficit	1.950 ± 0.3 Oxidative status is at a dangerous level in relation to cholesterol	142 ± 2.3 Oxidative status index is on the critical edge
Steam control	300 ± 2.27 Normal range	2840 ± 5.75 Slight deficiency	1.22 ± 0.2 Normal	40 ± 2.2 Normal
Experiment 5th day after radon exposure	381 ± 2.95 Is average ($p < 0.05$).	2091 ± 3.95 Slight deficiency	1.6 ± 0.2 High	111 ± 2.3 The body is on alert to protect itself

10th day after radon exposure	257±1.12 Normal range	2350±2.92 Normal value	0.96±0.001 Normal	31±2.3 Borderline
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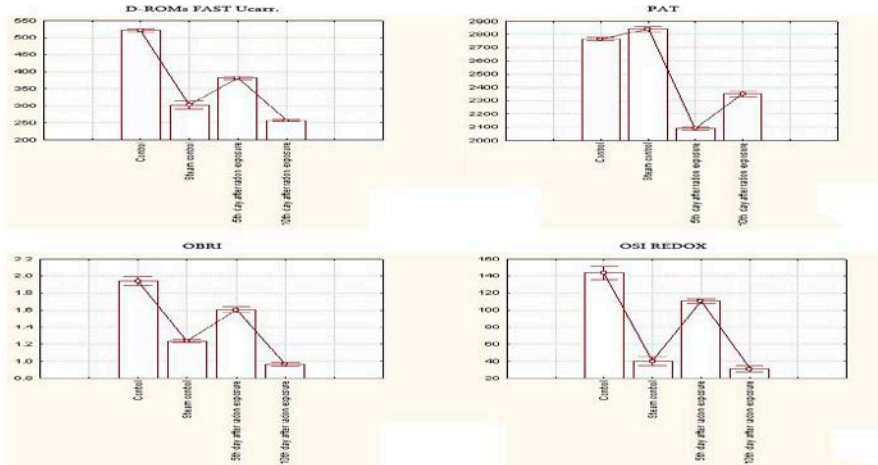


Figure 2. Oxidative stress in epileptic rats

Consequently, the difference between them is statistically significant as well ($p < 0.05$). OSI - The correlation of total oxidative status with the total antioxidant status used to determine the Global Redox status index in epileptic rats was 142 ± 2.3 before radon inhalation, which was above the critical situation. After inhalation it was halved to 111 ± 2.3 .

As for the 10th day after radon inhalation, all oxidative stress relievers approached the norm. From the above mentioned, it can be concluded that a positive result was obtained when inhaling radon.

Oxidative stress plays an important role in the biology of epilepsy (Ryan, 2011). Sulf groups acts like antioxidants and break chain of oxygen-derived free radicals. Measurements of serum thiol (Sulfhydryl group - SH), oxidative stress markers, which prevents the development of brain disorders associated with peroxidation reactions, is also used to evaluate the antioxidant status in case of epilepsy. As it is known from the literature, in case of disturbance of homeostasis caused by oxidative stress, the body tries to restore the disturbed homeostasis and activate the antioxidant systems by activating antioxidant systems, including Sulfhydryl groups. (Yarmoshenko, 2002; Zharkinbekova, 2019). Therefore, this results to the study of SH divided into protein thiols (PSH) and non-protein thiols (NPSH) on the 5th and 10th day after radon inhalation. This is because on the 10th day, the redox status was on the verge and it would be interesting to study sulfhydryl groups on the 5th -10th today.

Definition of Sulphydryl Groups

It is known from the literature that protein cysteine thiols respond to the cellular redox state. They can oxidize and inhibit thiol-proteins and enzymes and therefore have antioxidant action. In particular, when oxidants increase in the cell, thiol-disulfide is involved in redox regulation. These redox-sensitive mechanisms are involved in various changes including cell hypoxia. Under hypoxic conditions, the concentration of thiols decreases. This is due to the association of metabolites produced during the recovery of hypoxia with glutathione (GSH), a cellular nonprotein thiols (NPSH). This means the metabolites react with GSH instead of oxygen. When cellular thiols are depleted, peroxide is produced and excessive oxidative stress leads to cell death. Within the frames of our study, the quantitative variation of non-protein and total Sulphydryl groups was examined (Silva, 2011; Yuta Shibamoto, 2018). In doing this, on the 5th and 10th day after the end of the radon inhalation procedures, the concentration of non-protein and total SH groups in the rat brain was determined.

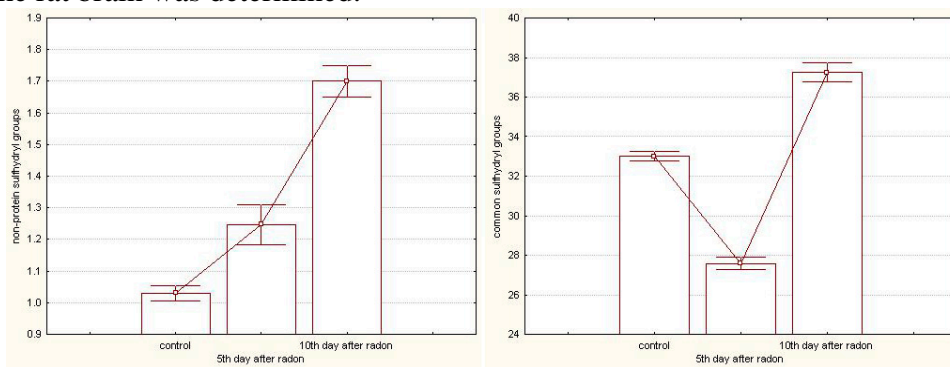


Figure 3. Sulphydryl amount (mcg / g in plasma M/m), ($p < 0.05$) in the control and experimental rat group on the 10th day after inhalation

Table 3. Non-protein and Common sulphydryl groups

	Control	Study group on the 5th day after radon inhalation	Study group on the 10th day after radon inhalation
Non-protein sulphydryl groups	$1,03 \pm 0,123$	$1,25 \pm 0,105^{**}$	$1,70 \pm 0,109^{**}$
Common sulphydryl groups	$33,00 \pm 1,22$	$27,56 \pm 1,41^{**}$	$37,16 \pm 1,44^{**}$

Note: $**P < 0.05$. $n = 28$ (14 in each group)

In the case of non-protein sulphydryl groups, on the 5th day (control 1.03 ± 0.123), it increased compared to the control and became $1.25 \pm 0.105^{**}$, while on the 10th day it became $1.70 \pm 0.109^{**}$. As for the total sulphydryl

groups, the concentration on the 5th day (control 33.00 ± 1.22) decreased compared to the control and became $27.56 \pm 1.41^{**}$ and on the 10th day compared to the control increased statistically and became $37.16 \pm 1.44^{**}$ (control 33.00 ± 1.22) respectively. Total sulfhydryl groups also had higher concentrations on the 10th day after radon inhalation compared to the control group 37.16 ± 1.44 and 1.70 ± 0.109 , respectively. Thus, this indicates an increase in the concentration of protein and total sulfhydryl groups on the 10th day compared to controls, and when compared to the group performing inhalation with conventional mineral water. The table shows the effect of radon inhalation on physiological processes, which acts as an activator or inhibitor of some neurotransmitters (Lee, 2018).

In view of all the above, it can be stated that radon exposure regulates oxidative stress, the clinical manifestation of which may manifest as a reduction in epileptic seizures, as evidenced by trials. Considering the fact that audiogenic epileptic seizures begin immediately after the call and last for minutes before inhalation of Tskaltubo in experimental rats, as shown in Figure 3, the duration of epileptic seizures does not exceed 2 seconds after inhalation of Tskaltubo water. Also, it was manifested without audiogenic seizures. Furthermore, it is also known that this enzyme, which is active in animals, consumes a large amount of ATP. Changes in glucose metabolism deficiency in the action of Na^+ / K^+ ATPase are associated with neuronal hyperactivity. This is one of the leading mechanisms to reduce the concentration of extracellular K^+ accumulated after convulsive activity. Low Na^+ / K^+ ATPase activity is associated with the development of epileptic seizures. In addition, Na^+ / K^+ ATPase activity was reduced within a few minutes after transient focal ischemia in rat cortex and hippocampus and in an experimental model of brain trauma. Altered ion homeostasis may also partially explain the interaction between convulsive activity and hypoglycemia (Hashemian, 2017). As can be seen from Table 4, there is an increase in Na^+ / K^+ ATPase on the 10th day after inhalation. It is important for reducing epileptic seizures and in some cases makes it disappear, as can be seen from previous experiments on the 3rd day after inhalation of Tskaltubo radon-containing water (Nikolashvili, 2006). In the following experiments, Na^+ / K^+ ATPase activity was studied on the 5th or 10th day after radon inhalation and, as the graph shows, the 5th day Na^+ / K^+ ATPase activity was 8.57 ± 0.10 mmol pi/mgE.houx. On the 10th day, the activity increased by 72%; but when compared with the 5th day, it increases by 174% which amounted to 14.89 ± 0.19 . Once again, this shows that on the 10th day after radon inhalation in Tskaltubo, there is a reduction in oxidative stress and it stays within the norm.

Na^+ / K^+ ATPase activity is manifested by a decrease in the first and second wild jogs in animals, including an increase in pause and a decrease in

tonic-clonic seizures. On day 10, it decreased and remained within 2 ± 1.1 seconds.

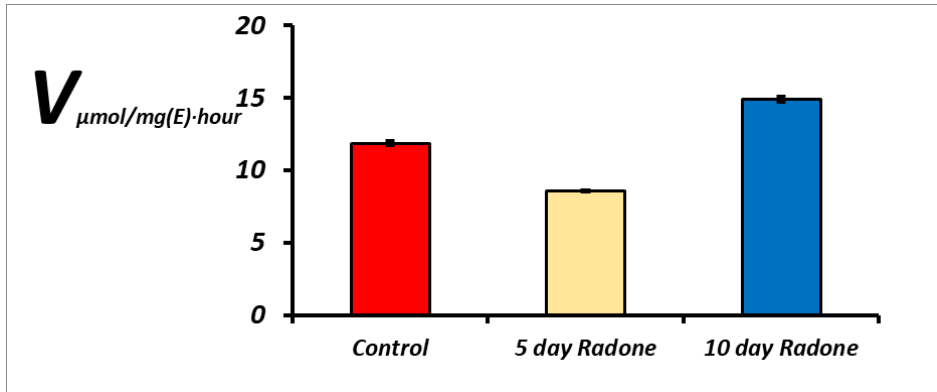


Figure 4. Rat brain plasma membrane Na,K-ATPase activity. Reaction medium: $[\text{MgATP} - \text{mmolpi/mgE houx}] = 1.69\text{mM}$; $[\text{ATP}_f] = [\text{Mg}^{2+}] = 0.31\text{mM}$ (MgATP – substrate)

Table 4. Na, K-ATPase activity

Research group	Na, K-ATPase activity	%
Day 5 after radon inhalation	$8,57 \pm 0,10$	100 %
On the 10th day after radon inhalation	$14,89 \pm 0,19$	174 % (Increased by 74% compared to the 5th day)

Thus, numerous experimental data and individual clinical observations indicate the pathogenic role of OS in epilepsy, which is associated with disruption of the structural hematoencephalic barrier integrity. It has also occurred based on the results of the study. According to this research, the use of radon inhalation in experimental animals, particularly in genetically determined rats with epileptic seizures, altered all parameters of the cell membrane of neurons, oxidative destruction of nucleic acids, and radon exposure that regulates oxidative stress. Also, the clinical manifestation may be expressed by a reduction in epileptic seizures. Some of the epileptic seizure development as shown in Figure 1 include: the hidden period and the first and second wild jog duration after the audiogenic signal. All what was mentioned suggests that radon inhalation could be used to treat epilepsy. Excretion of excitatory amino acids (mainly glutamate) is known to increase during seizures. At this time, neuronal activity increases and intracellular calcium levels increases also, and this is associated with the formation of ROS (Morandi, 2016). Disruption of calcium metabolism is associated with a sharp increase in cell cytosol, which leads to the activation of Ca^{++} dependent enzymes. The formation of ROS is accompanied by the synthesis of prostaglandins from arachidonic acid. However, this in turn enhances free radical reactions that ultimately lead to membrane destruction.

Thus, numerous experimental data and individual clinical observations indicate the pathogenic role of OS in epilepsy, which is associated with the disruption of the structural hemato-encephalic barrier integrity of the cell membrane of neurons, oxidative destruction of nucleic acids, and radon exposure that regulates oxidative stress. Also, the clinical manifestation may be expressed by a reduction in epileptic seizures which has also occurred based on the results of the study. According to our research, the use of radon inhalation in experimental animals, particularly in genetically determined rats with epileptic seizures, altered all parameters of the epileptic seizure development picture, namely the hidden period and the first and second wild jog duration after the audiogenic signal. All what was mentioned suggests that radon inhalation could be used to treat epilepsy. Based on the obtained data, it can be concluded that Inhalation of Tskaltubo water 37bk or 1 nk develops the effect of Hormesis, which causes positive changes in all the above oxidative stress markers in the brain as shown in this study. Based on the given data, inhalation of Tskaltubo water can be considered to be one of the methods of removing the convulsive effects and its treatment, which is confirmed by experimental studies.

Discussion

The data of this studies indicate that the effect of radon hormesis (R-Ho) happens by the activation of NO and formation of hydrogen peroxide (H_2O_2) that activate excessive NO production in microphages under the effect of interferon gamma and beta ($INF-\gamma\beta$) (7,9). As a result, the increased production of NO activates peripheral microcirculation and central hemodynamic (Marshalick, 1991). For this reason, NO can be considered as an autocrine homeostatic modulator as well.

It is established that macrophage-killers are the important source of NO. NO activation suppresses the DNA synthesis of tumor cells, new tumor cells, anti-inflammatory, desensibility, and sedative mechanisms. NO effects on activation of DOFA, DOFA-cines, and DOFA-amino formation in blood is involved in the regulation of the Na^+ , K^+ , and Ca^{++} ion changes. In addition, it plays one of the main roles in the suppression of specific autoimmune and in the activation of non-specific immune systems of the body (Hashemian, 2017; Isaksen, 2016).

In our case, the reduction in oxidative stress is seen as early as the 5th day after exposure to radon inhalation due to NO activation. NO can be considered as an important neurotransmitter that can participate in synaptic transmission as classical from presynaptic to postsynaptic neuron (Khazaradze, 2001; Jobe, 1973; Lomidze, 2012; Marshalick, 1991) and retrograde ISO indirectly by acting on glial cells or surrounding neurons. NO protects the brain from ischemic and neurotoxic stroke and controls the

oscillatory activity of neurons (Khazaradze, 2001; Krushinsky, 1970; Silva, 2011). Nitric oxide synthesis is often considered as a protective mechanism against the cytotoxic action of phagocytes, since NO inhibits the activation of the neutrophil (Nikolashvili, 2021). NADPH oxidase activity, reduces xanthine oxidase activity, and decreases AFK products (Pandey, 2012). At the same time, it should be noted that NO is involved in the development of the inflammatory process and its effect on the functional state of phagocytes can be modulated and changed over time. Therefore, the protective or cytotoxic action of NO is also a characteristic of certain cells and tissues. Experimental models of epilepsy show that increased oxidative stress (increased oxidation of lipids and proteins) is accompanied by the development of seizures (Gelashvili, 2001; Raimondo, 2015).

Studies by E. Ben-Menachem (2000) have shown that erythrocyte SOD1 activity is significantly lower in patients with progressive myoclonic epilepsy than in healthy individuals. Decreased SOD1 activity also occurs in the cerebrospinal fluid of patients with epilepsy, especially in the disease-resistant group compared with patients with curable forms of epilepsy and in the control group of healthy individuals. The authors believe that decreased SOD1 activity is associated with recurrent seizures, and that SOD1 deficiency in cerebrospinal fluid may be a predictor of drug-resistant epilepsy (Shultz, 2014).

In addition to the above, the convulsive state is characterized by anomalous Na^+ and K^+ metabolism. Accumulation of ammonia also occurs at this time, which is associated with the intensification of deamination reactions. All of this leads to depolarization of cell membrane shells, lowering of the excitability threshold, and a new series of seizures. In the first minutes of seizures, neurotransmitters are released and were accompanied by a change in the level of secondary messengers. Thus, this is reflected in the activity of metabotropic receptors. On the 5th and 10th day after radon inhalation, activation of adrenoceptors causes an increase in cyclic adenosine monophosphate. Inhalation of radon, as shown from previous studies, reduces the number of duration of behavioral seizures and in some cases makes them disappear (Morandi, 2015).

Excretion of excitatory amino acids (mainly glutamate) is known to increase during seizures. At this time, neuronal activity increases and intracellular calcium levels also increases, which is associated with the formation of ROS (Aguiar, 2012; Lomidze, 2012; Morandi, 2015). Disruption of calcium metabolism is associated with a sharp increase in cell cytosol, and this leads to the activation of Ca^{++} -dependent enzymes. The formation of ROS is accompanied by the synthesis of prostaglandins from arachidonic acid. This in turn enhances free radical reactions that ultimately leads to membrane destruction (Sedlak, 1968; Shultz, 2014; Yuta Shibamoto, 2018).

Thus, numerous experimental data and individual clinical observations indicate the pathogenic role of OS in epilepsy, which is associated with disruption of the structural hematoencephalic barrier integrity of the cell membrane of neurons, oxidative destruction of nucleic acids, and radon exposure that regulates oxidative stress. Also, the clinical manifestation may be expressed by a reduction in epileptic seizures which has also occurred based on the results of the study (Yarmoshenko, 2002; Yuta Shibamoto, 2018).

According to our research, the use of radon inhalation in experimental animals, particularly in genetically determined rats with epileptic seizures, altered all parameters of the epileptic seizure development picture, namely the hidden period and the first and second wild jog duration after the audiogenic signal. On the third day, no response to the audiogenic signal was observed at all, and there was not even a single episode of tonic-clonic seizures. All what was mentioned suggests that radon inhalation could be used to treat epilepsy (Bangasser, 2014; Yuta Shibamoto, 2018).

According to the International Classification of Epileptic Seizures on the study of focal seizures and the experiment carried out, the effectiveness of Tskaltubo water has been confirmed in the case of focal epileptic seizures. As for generalized epilepsy, the impact of radon hormone on these types of seizures is still unclear, which requires additional scientific studies (Hashemian, 2017).

The impact of radon inhalation on seizures of brainstem epilepsy models is particularly important because mechanisms of prolonged bilateral (formerly generalized) seizures in humans are considered to be erased/included in brainstem structures (Gelashvili, 2001; Isaksen, 2016).

The study is the first precedent of attempting R-Ho through inhalation for the treatment of epileptic seizures in animal models with further translation to clinical study in humans through pilot phase II study. More profound and scientifically systematized approach is needed to determine the uniqueness of Tskhaltubo water springs, investigation of the mechanisms of radon effects on the excitatory and inhibitory functioning of CNS, and further clinical studies to establish its effectiveness on humans (Gelashvili, 2001; Khazaradze, 2001).

Conclusion

Future research is required to clarify the mechanism of radon's action on antioxidative processes. However, based on the results of the experiment, the following conclusion was drawn:

Studies in experimental animals have shown that inhalation of Tskaltubo water develops a hormesis that regulates oxidative processes in the brain by activating antioxidants, which is reflected in the reduction of existing epileptic convulsions and in the activation of Na / K ATPase.

Inhalation of Tskaltubo water may be considered as a method of treatment with anticonvulsant effect confirmed by experimental studies.

References

1. Aguiar, CC., Almeida, AB., Araújo, PV., et al. (2012). Oxidative stress and epilepsy: literature review. *Oxid Med Cell Longev.*795259. doi:10.1155/2012/795259.
2. Bangasser, DA., & Valentino, RJ. (2014). Sex differences in stress-related psychiatric disorders: neurobiological perspectives. *Front Neuroendocrinol.* 35:303–19. doi: 10.1016/j.yfrne.2014
3. Bazzigaluppi, A.E. (2017). Hungry Neurons: Metabolic Insights on. international journal of molecular sciences, 28 October 4-5.
4. DiNuzzo, M., Mangia, S., Maraviglia, B., & Giove, F. (2014). Physiological bases of the K⁺ and the glutamate/GABA hypotheses of epilepsy. *Epilepsy Res.* 108, 995–1012.
5. Hashemian, M., Anissian, D., Ghasemi-Kasman, M., Akbari, A., Khalili-Fomeshi, M., Ghasemi, S. et al. (2017). Curcumin-loaded chitosan-alginate-STPP nanoparticles ameliorate memory deficits and reduce glial activation in pentylenetetrazol-induced kindling model of epilepsy. *Prog Neuropsychopharmacol Biol Psychiatry.* 79:462–71.
6. Isaksen, T.J., & Lykke-Hartmann, K. (2016). Insights into the pathology of the α 2-Na⁺/K⁺-ATPase in neurological disorders; lessons from animal models. *Front. Physiol.* 7, 161.
7. Gietzen, DW., Ross-Inta, CM., Rudell, JB., Hao, S., & Donovan, M. (2016). Calcium-dependent eukaryotic initiation factor 2 α phosphatase complexed with calcineurin restores control to anterior piriform cortex neural circuitry after activation by essential amino acid deficiency. *J Nutr Biol;* 2:33–44.5,
8. Gelashvili, GK., Vepkhvadze, N., Xazaradze, R., et al. (2001). Current radiation safety issues in Georgia. 1 National Conference. Tbilisi pp 146-156.
9. Jobe, PC., Picchioni, AL., & Chin, L. (1973). Role of norepinephrine in audiogenic seizure in the rat. *J. Pharmacol. Exp. Ther.* 184, 1-10.
10. Kaplan, D.I., Isom, L.L., & Petrou, S. (2016). Role of sodium channels in epilepsy. Cold Spring Harb. Perspect. Med.
11. Khazaradze, K.R., Khazaradze, R.E., Amiranashvili, A.E., Bliadze, T.A., Nodia, A.B., Nikiforov, G.I., & Chelidze, L.D. (2001). Some results of studies of radon content in the human environment // 1st international conference on ecology and environmental management of the Caucasus / Tbilisi, pp.61-63.

12. Krushinsky, L., Molodkina, L., Fless, D., Dobrokhotova, L., Steshenko, A., Zorina, Z., & Romanova, L. (1970). The functional state of the brain during sonic stimulation. – In: Welch B. and Welch A. (eds.). *Physiological effects of noise*, New York: Plenum Press, pp.151-158.
13. Lee, Y., Rodriguez, OC., Albanese, C., Santos, VR., Cortes de Oliveira, JA., Donatti, ALF., et al. (2018). Divergent brain changes in two audiogenic rat strains: a voxel-based morphometry and diffusion tensor imaging comparison of the genetically epilepsy prone rat (GEPR-3) and the Wistar Audiogenic Rat (WAR). *Neurobiol Dis.* 111:80–90. doi: 10.1016/j.nbd.2017.12.014
14. Lomidze, G., Kasradze, S., Kvernadze, D., Okujava, N., Toidze, O., Hanneke, M., de Boer, Tarun Dua, & Josemir W. Sander (2012). The prevalence and treatment gap of epilepsy in Tbilisi, Georgia, *Epilepsy Research*, Volume 98, Issues 2–3, Pages 123-129, ISSN 0920-1211.
15. Martin, K., Jackson, CF., Levy, RG., Cooper, PN. (2016). Ketogenic diet and other dietary treatments for epilepsy. *Cochrane Database Syst Rev* ; 2:CD001903
16. Morandi, G., Guido, D., & Tagliabue, A. (2015). A bibliometric study of scientific literature on the dietary therapies for epilepsy in Scopus. *Nutr Neurosci*;18:201–9.
17. Marshalick, BE., & Fenko, AN. (1991). The use of radon baths for rehabilitating the immune system of patients with bronchial asthma. *VoprKurortol Fiziother LechFiz Kult.* 1991;6:6–10.
18. Nikolashvili, M., Mchedluri, T., Museliani, T. (2006). Effect of tsvaltubo radon mineral waters on aggressive behaviour of animals and distribution of free amino acids in structures of the brain. *saq. mecn. Acad. Mmacne, ser. Bboil. A t 32, N1.* 119-123.
19. Nikolashvili, M., Nanobashvili, Z., & Mitagvaria, N. (2021). Radon hormesis in epileptic pathogenesis and predictors of oxidative stress *Georgian Medical News*, No 4 (313).
20. Pandey, M.K., Mitra, P., & Maheshwari, P.K. (2012). The Lipid Peroxidation Product as a Marker of Oxidative Stress in Epilepsy.
21. Raimondo, J.V., Burman, R.J., Katz, A.A., Akerman, C.J. (2015). Ion dynamics during seizures. *Front. Cell. Neurosci.*, 9, 419.
22. Ryan, MJ., Jackson, JR., Hao, Y., & Leonard, SS., Always, SE. (2011). Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. *Free Radic Biol Med.*; 51(1):38-52. doi:10.1016/j.freeradbiomed.2011.04.002

23. Ross, K.C., & Coleman, J.R. (2000). Developmental and genetic audiogenic seizure models: behavior and biological substrates. *Neuroscience and Biobehavioral Reviews*, 2000, 24, 639-653
24. Shultz, S.R., O'Brien, T.J., Stefanidou, M., & Kuzniecky, R.I. (2014). Neuroimaging the epileptogenic process. *Neurother. J. Am. Soc. Exp. Neurother.* 11, 347–357
25. Silva, L.F., Hoffmann, M.S., Rambo, L.M., Ribeiro, L.R., Lima, F.D., Furian, A.F., Oliveira, M.S., Figuera, M.R., & Royes, L.F. (2011). The involvement of Na⁺/K⁺-ATPase activity and free radical generation in the susceptibility to pentylenetetrazol-induced seizures after experimental traumatic brain injury. *J. Neurol. Sci.* , 308, 35–40.
26. Sedlak, J., & Landsay, R. (1968). *Biochem.* 25, 192-205.
27. Yarmoshenko, IV. (2002). Meta-analysis of 18 radon and lung cancer case control studies. In: Proceedings of the 7th International Symposium, Natural Radiation Environment (NRE VII), Rhodes, May, Elsevier Conf. Series, in press
28. Yuta Shibamoto & Hironobu Nakamura (2018). Overview of Biological, Epidemiological, and Clinical Evidence of Radiation Hormesis. *Int J Mol Sci.*; 19, (8):2387
29. Zharkinbekova, N.A., & Ormanov, N.Z. (2019). Lipid Peroxidation and the Antioxidant System in the Blood of Epileptic Patients. *Neurochem. J.* 13, 378–384.