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Late Changes in Oxidative Stress Biomarkers after Inhalation of Low Dose Radon in Young and Adult Wild Rats

Marine Nikolaishvili[*], Tea Museliani[*], Gogi Jikia[*], Aleksandre Tarkhnishvili[*], Khatuna Dondoladze[**]

[*] *Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*
[**] *Department of Behavioral Science, European University, Tbilisi, Georgia*

(Presented by Academy Member David Mikeladze)

Oxidative stress is involved in the development and progression of various diseases and is closely related to the aging process. Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify or neutralize them. These processes are accelerated by mitochondrial dysfunction, reduced antioxidant defence, inflammation, telomere shortening, DNA damage, etc. In the present work, the changes in the concentration of biomarkers of oxidative stress in young and old rats 3 months after inhalation of low doses of radon were studied. The hormetic mechanisms of radon therapy have not been thoroughly studied. However, exposure to low doses of radon enhances the mechanisms of antioxidant protection, and the results of our study show that under the influence of low doses of radon in adult rats, there is a change in the concentration of a number of biomarkers associated with oxidative stress, which is similar to the mechanisms of body "rejuvenation". In the group of young rats, the parameters also changed, although not to a statistically significant degree. As a result, the hormetic effect of small doses of radon, in particular inhaled low doses of radon, can be considered as so-called "antioxidant supplement". *© 2024 Bull. Georg. Natl. Acad. Sci.*

oxidative stress biomarkers, antioxidation, radon, aging

Oxidative stress is a condition when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify or neutralize them. ROS are natural by-products of cellular metabolism, highly reactive molecules that begin to participate in the reactions of various physiological mechanisms [1]. Several factors can contribute to the generation of ROS and the development of oxidative stress, including environmental toxins, pollutants, radiation, stress, certain medications, chronic inflammation, etc.

Oxidative stress is involved in the development and progression of various diseases, including cardiovascular disease, neurodegenerative disorders (e.g., Alzheimer's disease, Parkinson's disease), diabetes, tumors, and age-related senile degenera-

tion. Oxidative stress contributes to cellular dysfunction, tissue damage, and chronic inflammation.

Oxidative stress is known to be closely related to the aging process. Over time, the accumulation of toxins resulting from the oxidation of cellular components such as DNA, proteins, and lipids contributes to cell and tissue functional decline and increases susceptibility to age-related diseases. These processes are accelerated (especially in an aged body) by mitochondrial dysfunction, decreased antioxidant protection, inflammation, shortening of telomeres [2], DNA damage etc., which are manifested in the acceleration of aging processes.

The ratio of biomarkers of oxidative stress to antioxidants indicates the oxidative balance of the body, i.e., the ratio between the production of reactive oxygen species (ROS) and the antioxidant defense in the body: the redox balance, which is calculated by the ratio of biomarkers of oxidative stress to biomarkers of antioxidants.

The aim of our study is to study changes in the concentration of oxidative stress biomarkers in young and old rats 3 months after of low doses of radon inhalation.

The potential antioxidant effects of radon water, in particular the antioxidant processes associated with radon, are not well understood. Radon is primarily known to be a health hazard (especially with an increased risk of lung cancer in smokers), but it is important to note that a number of studies have demonstrated the potential beneficial therapeutic effects of radon therapy, which involves controlled exposure to low doses of radon during spa treatment or experimental studies.

These mechanisms of radon therapy have not been extensively studied and are difficult to understand, although exposure to low doses of radon can induce hormonal effects that potentially enhance antioxidant defense mechanisms and lead to beneficial health effects. To obtain a hormetic effect, it is necessary to take into account many factors: the state of the body, the concentration of radon, the duration and frequency of action, and others. It can be seen from experimental studies that the hormetic effect does not occur immediately with the action of radon, but after a certain period (e.g., 1-3 months) [3]. Therefore, we decided to study the effect of a low dose of radon on the oxidative and antioxidant systems 3 months after inhalation (late changes).

Materials and Methods

Study design: For the experimental group, we selected 7 young (3 months) and 7 adult (24 months) wild rats from the vivarium, which were placed for radon inhalation in the balneological treatment room of the resort center of Tskhaltubo (a city in Georgia known for its radonized waters). Together with the steam in this room, natural radonized water flows out of the ground. We measured the concentration of radon in the steam bath. Control group: 7 rats of the control group (12 months) were not subjected to radon therapy.

The rats were kept in the spa for 1-1 hour for 5 days. We took blood before the procedure and 3 months after the 5-day inhalation procedure. After the end of the study, we compared changes in the concentration of oxidative stress biomarkers before and after the procedure in two experimental (young and old) and control groups.

Radon Measurement: We measured the radon concentration in the Tskaltubo spa room, which was 37 becquerels (bk) per cubic meter (37 bk/m^3).

Assessment of oxidative and antioxidant biomarkers. Using the Photometric Analytical System – FRAS 5 [4], in the blood plasma of experimental and control rats, we measured the concentrations of free radicals (D-ROMs) – reactive oxygen metabolites by photometric test and the concentration of hydroperoxides (ROOH) in the brain tissue, which gives us a pro-oxidant status of the tissue, and to study the antioxidant capacity of the body, we determined the OSI (Oxidative Stress Index) and the OBRI (Oxidation Balance Status). For this, we used PAT (Antioxidant Concentration Test) by

measuring ferric reduction ability, and to evaluate the effectiveness of antioxidants.

For statistical analysis, we used Single Variable Data Analysis (one-way ANOVA). The results are presented (mean +/- standard error (SE), in statistical significance of evidence $p < 0.05$.

All studies are conducted in accordance with international GLP standards. All rat experimental protocols were approved by the Laboratory Animal Care and Use Committee. The principles of ethical treatment of animals were respected in accordance with Georgian legislation and international agreements.

Results and Discussion

We determined the normal limits of D-ROMs, PAT, OBRI and OSI-REDOX concentrations with oxidative and antioxidant biomarkers of blood plasma of control and young rats, which is presented in Figure. Note: PAT data is based only on the data of the younger group before radon inhalation.

In the experimental group of young and aged rats, we measured the concentration of biomarkers of oxidative and antioxidant systems before the radon inhalation procedure (see Table 1).

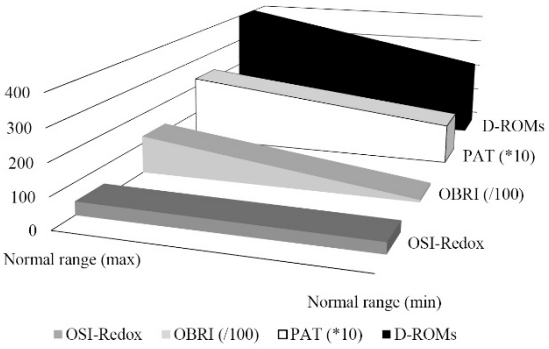

Fig. Standard (normal concentration) limits of biomarkers of oxidative and antioxidant systems.

The results of the study show that in the group of young rats, before radon inhalation, the concentration of the body's oxidative and antioxidant biomarkers is within the norm, i.e., compared to the oxidative and antioxidant biomarkers of the control group, no statistically significant difference ($p < 0.05$) was observed. D-ROMs, OBRI and OSI-REDOX – in that case, while a small but statistically significant deficiency of PAT (pro-oxidant anti-oxidant balance) biomarkers was detected in the control group ($p < 0.05$).

Biomarkers of oxidative and antioxidant systems were determined in blood plasma of young and adult rats 3 months after inhalation of small

Table 1. Biomarker concentrations of oxidative or antioxidant systems in young and adult rats before radon inhalation

Rats groups	D-ROMs FAST Ucarr.	PAT	OBRI	OSI REDOX
Control group	301±2.27 (normal range)	2442±5.75 (small deficiency)	1.22±0.2 (normal range)	42±2.2 (normal range)
Elderly group	535±3.67 (high level)	2488±5.85 (deficit)	1.8±0.3 (high level)	48±2.3 (high, critical level)
Young group	259±1.13 (normal range)	2324±2.82 (normal range)	0.9±0.001 (normal range)	38.5±2.1 (normal range)

Table 2. Concentrations of biomarkers of oxidative or antioxidant systems of young and adult rats 3 months after a 5-day radon inhalation procedure (in the control group, no inhalation procedure was performed)

Rats Groups	D-ROMs FAST Ucarr.	PAT	OBRI	OSI REDOX
Elderly group	454±1.07 (high level)	2446±4.43 (normal range)	1.5±0.51 (High level)	46±1.09 (High level)
Young group	248±0.99 (normal range)	2318±1.03 (normal range)	0.9±0.019 (normal range)	35.5±1.8 (normal range)

doses of radon (results are presented in Table 2). The obtained results show that in both groups the data changed and the antioxidant system improved, while the concentration of biomarkers of oxidative stress decreased, although only PAT returned to normal in the group of adult rats ($p < 0.05$). OSI decreased statistically significantly and did not deviate from the critical limit, although it remained high compared to the norm.

The results of our studies show that when adult rats were exposed to small doses of radon, the concentration of a number of biomarkers associated with oxidative stress changed, which is similar to the mechanisms of body "rejuvenation" [5]. In the group of young rats, the parameters also changed, although not to a statistically significant degree. As a result, the hormetic effect of small doses of radon, in particular small doses radon inhalation, can be considered the so-called "as an antioxidant supplement". It is known that certain antioxidant supplements or manipulations, medical treatments, healthy lifestyle, antioxidant-rich foods, exercise, stress management and avoidance of exposure to environmental toxins can help to reduce oxidative stress and slow the rate of aging.

There is a complex and multifaceted relationship between oxidative stress and aging. While excessive oxidative stress may contribute to age-related disease and functional decline, some level of oxidative stress is a natural part of cellular function and signaling, although a balance between oxidative and antioxidant defense mechanisms is important for healthy aging.

Our study shows a specific positive health effect of Tskaltubo mineral water that may have additional benefits, namely the hormetological effect of radon, which supports the hypothesis that exposure to low doses of radon gas stimulates beneficial reactions in the body potentially leading to improve one's health.

Conclusion

According to the results of the study, changes in the biomarkers of the oxidative and antioxidant systems were observed 3 months after low doses of radon inhalation. The changes are especially pronounced in the adult body, although the hormetic effect of radon needs to be deeply studied.

ადამიანისა და ცხოველთა ფიზიოლოგია

ოქსიდაციური სტრესის ბიომარკერების მოგვიანებითი ცვლილება რადონის მცირე დოზების ინჰალაციისას ახალგაზრდა და ზრდასრულ უჯიშო ვირთაგვებში

მ. ნიკოლაიშვილი*, თ. მუსელიანი*, გ. ჯიქია*, ა. თარხნიშვილი*, ხ. დონდოლაძე**

* *ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, რადიო-ბიოლოგიის ლაბორატორია, თბილისი, საქართველო*
** *ევროპის უნივერსიტეტი, ქცევათმეცნიერების დეპარტამენტი, თბილისი, საქართველო*

(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

ოქსიდაციური სტრესი მონაწილეობს სხვადასხვა დაავადების განვითარებასა და პროგრესირებაში, იგი მჭიდრო კავშირშია დაბერების პროცესთან. ოქსიდაციური სტრესი არის დისბალანსი რეაქტიული ჟანგბადის სახეობების (ROS) გამომუშავებასა და ორგანიზმის დეტოქსიკაციის ან მათი განეიტრალების უნარს შორის. ამ პროცესებს კი აჩქარებს მიტოქონდრიული დისფუნქცია, ანტიოქსიდანტური დაცვის დაქვეითება, ანთება, ტელომერების შემცირება, დნმ-ის დაზიანება და სხვა. წარმოდგენილ ნაშრომში შევისწავლეთ ოქსიდაციური სტრესის ბიომარკერების კონცენტრაციის ცვლილებები ახალგაზრდა და ასაკოვან ვირთაგვებში რადონის მცირე დოზებით ინჰალაციიდან 3 თვის შემდეგ. რადონის თერაპიის ჰორმეზისული მექანიზმები საფუძვლიანად არ არის შესწავლილი, თუმცა რადონის დაბალი დოზით ზემოქმედებამ გააძლიერა ანტიოქსიდანტური დაცვის მექანიზმები და კვლევის შედეგებში ჩანს, რომ რადონის მცირე დოზების მოქმედებისას ზრდასრულ ვირთაგვებში მოხდა ოქსიდაციურ სტრესთან დაკავშირებული რიგი ბიომარკერების კონცენტრაციის ცვლილება, რაც ორგანიზმის „გაახალგაზრდავების“ მექანიზმების მსგავსია. ახალგაზრდა ვირთაგვების ჯგუფშიც შეიცვალა პარამეტრები, თუმცა სტატისტიკურად სარწმუნო მოცულობის გარეშე. შედეგად, რადონის მცირე დოზის ჰორმეზისული ეფექტი, კერძოდ, რადონის მცირე დოზების ინჰალაცია შესაძლებელია განვიხილოთ ე.წ. „ანტიოქსიდანტურ დანამატად“.

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