საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 18, №2, 2024
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 18, no. 2, 2024

Human and Animal Physiology

Oxidative and Antioxidative Activity of Organism after Conditions of Hyperthermia-Induced Hormesis and its Disruption by High Temperature

Marina Devdariani[*], Babry Oren[**], Mariam Darbaidze[§], Nino Sikharulidze[*], Ia Kvachakidze[*], Marina Nebieridze[*], Lena Davlianidze[*], Lali Gumberidze[*], Tengiz Zaalishvili[§§], Nodar Mitagvaria[§§]

[*] *Laboratory of Cerebral Circulation and Metabolism, Ivane Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia*
[**] *BAO Health Resource Corporation, Tarzana, USA*
[§] *Alter Bridge University, Tbilisi, Georgia*
[§§] *Academy Member, Georgian National Academy of Sciences, Tbilisi, Georgia*

The whole-body hyperthermia in a certain temperature range leads to the emergence of the phenomenon of hormesis, which is one of the strongest natural defense reactions of the body, the fundamental basis for understanding of which is "dose-response". Our data indicate that the dose of oxidative stress induced by 40°C whole-body hyperthermia was in the range required to stimulate hormetic mechanisms. We hypothesize that the main reactive oxygen species that trigger the activation of the hormetic mechanism are superoxide and hydrogen peroxide. High-level (45°C) hyperthermia-induced hormesis disruption in rats showed an increase in the concentration of free radicals (d-ROMs) and oxidative stress index (OSI), indicating the presence of high levels of oxidative stress. In addition to superoxide, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite are also important. We observed a disruption of the hormesis phenomenon, showing a positive effect at low doses of the stressor and a negative effect at high doses. According to the obtained results, in the case of whole-body hyperthermia (WBH), the temperature should not exceed 40°C, because the further increase in temperature leads to increased aggregation of red blood cells and deterioration of blood viscosity, which leads to rapid slowing of blood flow, impaired glucose supply to the tissue. Thus, whole-body hyperthermia of 40°C can be used as a "trigger" to activate hormetic mechanisms. When we use WBH in oncological or other types of studies, it is fundamentally important that the temperature interval of hyperthermia to be within the "hormetic range" not only for the efficiency of the hormetic mechanism, but also to maintain blood rheological indicators within the norm.

oxidative stress, whole body hyperthermia, reactive oxygen radicals, antioxidants

The resistance of living organisms to stress is one of the most important indicators of its viability, and it is clear that the study of the mechanisms that form this resistance is fundamentally important. A large

number of experimental studies have shown that oxidative stress (a condition in which the balance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell is disturbed) can be responsible to varying degrees for some diseases (cancer, diabetes, Alzheimer's, Parkinson's, autism, cardiovascular diseases) onset and/or progression, but it can also play an important role in the regulation of physiological adaptation, redox-homeostasis of cells and intracellular signal transmission. Reactive oxygen species (ROS) are mainly produced by mitochondria under both physiological and pathological conditions, superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), nitric oxide (NO•), etc., are considered reactive oxygen species (ROS). They are produced as metabolic by-products by biological systems [1,2]. Processes such as protein phosphorylation, activation of several transcription factors, apoptosis, immunity, differentiation depend on the correct generation of ROS in cells and their maintenance at a low level [3]. As the generation of ROS increases, they start to have harmful effects on important cellular structures [4]. Mitochondria have been shown to be the main source of ROS in response to hyperthermia-induced oxidative stress, and the main reactive oxygen species is superoxide. Heat stress increases mitochondrial superoxide levels [5,6]. In addition, hyperthermia-induced oxidative stress leads to overproduction of transition metal ions (TMI), which can donate electrons to oxygen to form superoxide anions [7,8]. Superoxide anions can be converted to hydrogen peroxide (H_2O_2) either spontaneously or via a reaction catalyzed by the SOD enzyme. Superoxide can react with other radicals including NO, the resulting product, peroxynitrite ($ONOO^-$), is a very strong oxidant [9,10]. Compared to other free radicals, due to thier small size and relatively benign reactivity, H_2O_2 can freely diffuse across cell membranes. Thus, it can mediate toxic effects far from the site of ROS generation. Heat stress has been shown to increase the production of H_2O_2 [11,12] and hydroxyl radicals (OH•) [13]. The extremely reactive hydroxyl radical OH• is formed from H_2O_2 through the Fenton reaction, which has a very short half-life and reacts with any nearby molecule [14].

Cells use an antioxidant defence system mainly based on enzymatic components such as superoxide dismutase (SOD), catalyse (CAT) and glutathione peroxidase (GPx) to protect themselves from cellular damage caused by ROS.

It should be emphasized that if oxidative stress is not strictly controlled, it can be responsible for several diseases, both chronic and degenerative, as well as for accelerating the aging process of the body and causing acute pathologies (e.g, trauma and stroke).

The main tasks of our research were as follows: 1. Precise identification of the safe temperature range for normal tissue during WBH exposure; 2. Quantitative study of oxidative (ROMs) and antioxidant (PAT) statuses developed in the body under conditions of oxidative stress of different strength induced by WBH exposure.

Material and Methods

The study was conducted on groups of white laboratory rats (following international principles of handling them). Animals were subjected to whole body hyperthermia (WBH) through a hypothermic cabin in the laboratory, in which the animals were not restricted in their freedom of movement, drinking water and food supply. Different temperature regimes were used: low (38°, 39°, 40°C), moderate (41°, 42°, 43°C) and high (44°, 45°C). For each of these regimens, the presence and duration of the hormetic range was examined.

FRAS 5 analyzer (Italy, H&D) was used to determine the amount of free radicals and the antioxidant status of the body, which allows to quantitatively measure the concentration of free radicals in the Carratelli Unit (1U.Carr=0.08 mg H_2O_2/dl), and the concentration

of antioxidants in the Cornelli Unit (1U.Cor=1.4 µmol/L of ascorbic acid).

Results and Discussion

Before discussing the test results, we would like to mention some important facts regarding the change of brain temperature in relation to temperature changes in the hypothermic cabin (HC), namely the phenomenon of autoregulation of brain temperature. The fact is that if we increase the temperature in HC to 44-45°C, the temperature in the brain tissue does not change and remains in the range of about 36-36.5°C. However, further increase in temperature in the hypothermic cabin causes the temperature in the brain to rise and when the brain temperature reaches about 41°C, the animal dies. Thus, in the case of exposure to 40-44°C in a hypothermic cabin, the brain temperature of test animals does not change dramatically even if the WBH exposure lasts for several hours.

The data obtained as a result of the study of the oxidative and antioxidant status developed under WBH conditions are presented below in the form of graphs (Fig. 1-3).

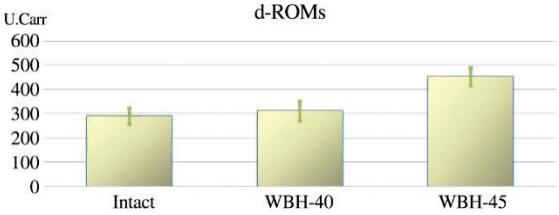

Fig. 1. D-Rom number of free radicals – under normal, 40°C and 45°C hyperthermia conditions.

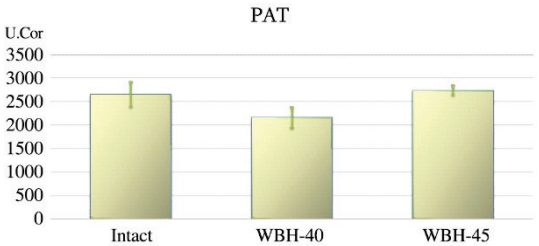

Fig. 2. The amount of plasma antioxidants – under normal, 40°C and 45°C hyperthermia conditions.

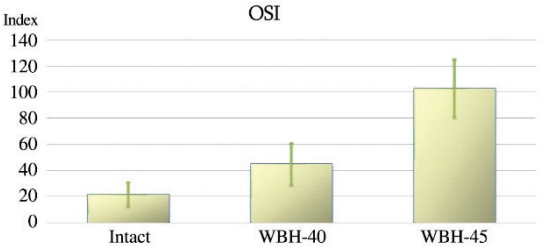

Fig. 3. Oxidative stress index – under normal, 40°C and 45°C hyperthermia conditions.

As can be seen from the obtained data, there is an increase in the number of free radicals (d-ROMs) and the oxidative stress index (OSI) during 40°C and 45°C WBH exposure. As for the amount of antioxidants, it varies within the norm under the conditions of both thermal regimes. The dose of oxidative stress induced by 40°C whole body hyperthermia was in the range required to stimulate hormetic mechanisms. In our opinion, the main reactive oxygen species that trigger the activation of the hormetic mechanism are superoxide and hydrogen peroxide.

High-level (45°C) hyperthermia-induced hormesis disruption in rats resulted in increased free radical counts (ROMs) and oxidative stress index (OSI), indicating high levels of oxidative stress. In addition to superoxide, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite are also important. Therefore, we have seen a violation of the phenomenon of hormesis, which shows a positive effect with low doses of the stressor and a negative effect with high doses.

According to our results, in case of hyperthermia of the whole body, the temperature should not exceed 40°C, because the increase in temperature leads to the increase of red blood cell aggregation and the deterioration of blood viscosity, which leads to a rapid slowing of blood flow, disruption of glucose supply to the tissue. 40°C still allows blood circulation throughout the body to be maintained at a normal level (the hormetic effect is in effect) and all the other benefits of hypothermic exposure are still in effect.

Thus, through experiments conducted on white laboratory rats, under conditions of whole-body hyperthermia (WBH), the so-called "hormetic range" of oxidative stress was determined, within which immune protection mechanisms are activated in the body and its disorders are effectively eliminated.

Conclusion

Thus, stimulation of the hormetic mechanism can be used to adapt to various types of stressors and successfully treat many pathological processes (including cancer).

Finally, when we use whole-body hyperthermia as a "trigger" for hormesis, in oncological or other studies, it is fundamentally important that the temperature interval of hyperthermia be within the "hormetic range", not only for the efficiency of the hormetic mechanism, but also to maintain blood rheological parameters within the normal range.

This paper needs further study.

ადამიანისა და ცხოველთა ფიზიოლოგია

ორგანიზმის ოქსიდანტური და ანტიოქსიდანტური აქტივობა ჰიპერთერმიით გამოწვეულ ჰორმეზისის პირობებსა და მისი მაღალი ტემპერატურით დარღვევის შემდეგ

მ. დევდარიანი[*], ბ. ორენი[**], მ. დარბაიძე[§], ნ. სიხარულიძე[*], ი. ქვაჩაკიძე[*], მ. ნებიერიძე[*], ლ. დავლიანიძე[*], ლ. გუმბერიძე[*], თ. ზაალიშვილი[§§], ნ. მითაგვარია[§§]

[*] *ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თავის ტვინის სისხლის მიმოქცევისა და მეტაბოლიზმის ლაბორატორია, თბილისი, საქართველო*
[**] *BAO Health Resource Corporation, ტარზანა, აშშ*
[§] *ალტერბრიჯის უნივერსიტეტი, თბილისი, საქართველო*
[§§] *აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია, თბილისი, საქართველო*

მთელი სხეულის ჰიპერთერმია გარკვეულ ტემპერატურულ დიაპაზონში იწვევს ჰორმეზისის ფენომენის აღმოცენებას, რაც წარმოადგენს ორგანიზმის ერთ-ერთ უძლიერეს ბუნებრივ თავდაცვით რეაქციას, რომლის გაგების ფუნდამენტური საფუძველია „დოზა-რეაქცია". ჩვენ მიერ მიღებული მონაცემები მიუთითებს იმაზე, რომ 40°C-იანი მთელი სხეულის ჰიპერთერმიით ინდუცირებული ოქსიდაციური სტრესის დოზა იყო ჰორმეზული მექანიზმების სტიმულაციისთვის საჭირო დიაპაზონში. ვვარაუდობთ, რომ ძირითადი რეაქტიული ჟანგბადის

სახეობა, რომელიც იწვევს ჰორმეზული მექანიზმის გააქტიურებას, არის სუპეროქსიდი და წყალბადის ზეჟანგი. მაღალი დონის (45°C) ჰიპერთერმიით გამოწვეული ჰორმეზისის მექანიზმის დარღვევის პირობებში ვირთაგვებში ადგილი ჰქონდა თავისუფალი რადიკალების (d-ROMs) კონცენტრაციისა და ოქსიდაციური სტრესის ინდექსის (OSI) მატებას, რაც მიუთითებს მაღალი დონის ოქსიდაციური სტრესის არსებობაზე. სუპეროქსიდის, ჰიდროქსილის რადიკალის, წყალბადის ზეჟანგის გარდა, ასევე მნიშვნელოვანია აზოტის ოქსიდი და პეროქსინიტრიტი. ჩვენ დავინახეთ ჰორმეზისის ფენომენის დარღვევა, რომელიც აჩვენებს პოზიტიურ ეფექტს სტრესორის დაბალი დოზებით და უარყოფით ეფექტს მაღალი დოზებით. მთელი სხეულის ჰიპერთერმიის შემთხვევაში (WBH) მიღებული შედეგების მიხედვით ტემპერატურა არ უნდა აღემატებოდეს 40°C-ს, რადგან ტემპერატურის შემდგომი მატება იწვევს სისხლის წითელი უჯრედების აგრეგადობის გაზრდას და სისხლის სიბლანტის გაუარესებას, რაც იწვევს სისხლის ნაკადის სწრაფ შენელებას, ქსოვილში გლუკოზის მიწოდების დარღვევას. ამრიგად, 40°C–იანი მთელი სხეულის ჰიპერთერმია შეიძლება გამოყენებული იყოს, როგორც „გამშვები ტრიგერი“ ჰორმეზული მექანიზმების ამოქმედებისათვის. როდესაც ჩვენ ვიყენებთ WBH–ს ონკოლოგიურ, ან სხვა სახის კვლევებში, პრინციპულად მნიშვნელოვანია, რომ ჰიპერთერმიის ტემპერატურული ინტერვალი იყოს „ჰორმეზული დიაპაზონის“ ფარგლებში არა მარტო ჰორმეზული მექანიზმის ეფექტუროზისთვის, არამედ სისხლის რეოლოგიური მაჩვენებლების ნორმის ფარგლებში შესანარჩუნებლად. წინამდებარე ნაშრომი საჭიროებს დამატებით კვლევას.

REFERENCES

1. Sato H., Shibata H., Shimizu T., Shibata S., Toriumi H., Ebine T. (2013) Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. *Neuroscience*, 248:345–358.
2. Navarro-Yepes J., Zavala-Flores L., Anandhan A., Wang F., Skotak M., Chandra N. (2014) Antioxidant gene therapy against neuronal cell death. *Pharmacology & Therapeutics*, 142:206–230.
3. Rajendran P., Nandakumar N., Rengarajan T., Palaniswami R., Gnanadhas E. N., Lakshminarasaiah U. (2014) Antioxidants and human diseases. *Clinica Chimica Acta*, 436:332–347.
4. Wu J. Q., Kosten T. R., Zhang X.Y. (2013) Free radicals, antioxidant defense system, and schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 46: 200-206.
5. Mujahid A., Pumford NR., Bottje W., Nakagawa K., Miyazawa T., Akiba M. et al. (2007) Mitochondrial oxidative damage in chicken skeletal muscle induced by acute heat stress. *J Poult Sci.,* 44: 439-45.
6. Mujahid A., Sato K., Akiba Y., Toyomizu M. (2006) Acute heat stress stimulates mitochondrial superoxide production in broiler skeletal muscle, possibly via downregulation of uncoupling protein content, *Poult Sci.,* 85: 1259-65.
7. Freeman ML., Spitz DR., Meredith MJ. (1990) Does heat shock enhance oxidative stress? Studies with ferrous and ferric iron, *Radiat Res,* 124: 288-93.
8. Agarwal A., Prabhakaran SA. (2005) Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. *Indian J Exp Biol.,* 43: 963-74.
9. Beckman JS., Koppenol WH. (1996) Nitric oxide superoxide and peroxynitrite: the good the bad and the ugly. *Am J Physiol.,* 271: C1424-37.
10. Radi R., Cassina A., Hodara R., Quijano C., Castro L. (2002) Peroxynetrite reactions and formation in mitochondria. *Free Radic Biol Med.,* 33: 1451-64.
11. Kikusato M., Toyomizu M. (2013) Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. *PLoS One,* 8: e64412.
12. Zhao QL., Fujiwara Y., Kondo T. (2006) Mechanism of cell death induced by nitroxide and hyperthermia. *Free Radic Biol Med.,* 40: 1131-43.
13. Flanagan SW., Moseley PL., Buttner GR. (1998) Increased flux of free radicals in cells subjected to hyperthermia: detection by electron paramagnetic resonance spin trapping. *FEBS Lett.,* 431: 285-6.
14. Kirkinezos IG., Moraes T. (2001) Reactive oxygen species and mitochondrial diseases. *Cell Dev Biol.,* 12: 449-57.

Received May, 2024