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Human and Animal Physiology

Whole Body Hyperthermia Induced Phenomenon of Hormesis (Experimental Study)

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Hyperthermia is almost always used with other forms of cancer therapy, such as radiation therapy and chemotherapy. Hyperthermia makes some cancer cells more sensitive to radiation or harms other cancer cells that radiation cannot damage. The less is known concerning the possible effects of Whole Body Hyperthermia (WBH) on the processes of learning and memory, and on rheological properties of blood. These issues served as the main purpose of this work, but in the course of the study we had new challenges, which revealed the new unknown healing opportunities of the WBH. Experiments on laboratory rats (using hyperthermia chamber) behavioral tests using multi-branch maze showed the following effects: 1. WBH might be used as one of the most effective triggering factor for launching of Hormetic Phenomenon; 2. In all cases when you use WBH as a trigger for Hormetic Phenomenon in cancer or any other clinics, the critical is to make sure that the chosen level of temperature does not exceed the Hormetic range and that is very critical not just for action of Hormetic mechanisms, but also for maintenance of blood rheological properties at the normal levels.

Whole body hyperthermia, phenomenon of hormesis, cancer, blood rheological properties, behavioral tests, toxicity, white rats

The fundamental basis for understanding the Phenomenon of Hormesis is the "dose-response" [1], which shows the process of stimulation at lower doses and inhibition – at high. Low or high doses of stress factors cause, respectively, eustress or strong distress, resulting in activation of moderate or damaging allostatic buffering capacity of the organism. This is true no matter what the nature of the stressor is: physical, chemical or mental [2]. Hyperthermia is almost always used in combination with other forms of cancer therapy, such as radiation therapy and chemotherapy. It may make some cancer cells more sensitive to radiation or harm other cancer cells that radiation cannot damage [3]. Hyperthermia can also enhance the effects of certain anticancer drugs, which is mutually strengthening and makes healing more likely – the so-called synergistic effect of hyperthermia [4]. High temperature (40-44°C) can damage and kill cancer cells, usually with minimal injury to normal tissues [5] which are tolerant (with an exception of the central nervous cells) to

hyperthermic exposure and can survive at temperatures of up to 44^0C. The main tasks of our study were as follows: 1. Accurate identification of safety temperature range for normal tissue during the Whole Body Hyperthermia (WBH) exposure; 2. Obtaining of more accurate data of information concerning the influence of WBH on blood rheological properties and the severity of induced behavioral disturbances.

Methodical Approach

Two series of experimental study of effects of WBH on memory and learning skills of the test animals were carried out on the groups of white rats placed in a special hyperthermia chamber for one hour, without any restrictions of movement. Three heat settings (38, 39 and 40^0C) were used and the strategy of the experiments did not depend on the temperature. The first series – effect of WBH on memory: 1. A group of rats learn to determine the optimal path in a multi-branch maze before the hyper-thermal exposure and the process of learning lasts until the development of automatism in their behavior; 2. In order to determine the blood relative viscosity, aggregability of RBC and other rheological properties, blood samples were taken from 10% of the animals of these series; 3. Each animal underwent a one-hour hyperthermia exposure; 4. In order to determine the effect of WBH on memory the exposed animals were re-tested in the maze one hour after the hyper-thermal exposure, then one day later and, finally, one week after the exposure (the full passing time, number of errors committed during the passage of the maze were recorded); 5. On the second day after WBH exposure blood samples were once again taken from the animals (after completion of testing in the maze) to determine the blood relative viscosity, aggregability of RBC and other rheological parameters. The second series – effect of WBH on the process of learning. All three temperature groups of animals in this series, first of all, had to get the relevant hyperthermic exposure, and only after that (starting from the second day) they were trained in the multi-branch maze. Comparison of the received results with those of the first series allowed us to make conclusions about the effect of WBH on the learning process.

Behavioral Study of Learning and Memory Processes

A standard maze consisting of platforms (multi-branch maze) fixed on supports at the height of 30 cm was used. Undergoing the method of trial and error the rats learn to move along the optimal trajectory, depending on experimental conditions accomplishing this in several minutes to several seconds. Such a learning method was found to be most acceptable and fitted to the task at hand. In order to evaluate the effects of WHB on the learning and memory levels the recorded data were statistically evaluated and appropriate conclusions were made.

Blood Rheology

Determination of RBC Aggregability Index. Blood samples were centrifuged and about 0.1 ml of blood was diluted 1:200 in own plasma in the Thoma pipettes preliminary rinsed with 5% sodium citrate solution without addition of any other anticoagulants. After the standard mixing the diluted blood was placed into a glass chamber. The quantitative index of RBC aggregation, which can be assessed with a special program at the Texture Analysis System (TAS-plus, "Leitz, Germany), represented the ratio of the aggregated and non-aggregated RBC.

Blood plasma viscosity. Blood plasma viscosity was examined in the capillary viscometer. Diameter of the capillary is about 1.8 mm. Displacement of plasma samples is induced by the gravity force related to the difference of levels of the plasma under study (without application of an additional pressure). For evaluation of the plasma viscosity in centipoises (cp) we determined the

calibration factor (F). Blood plasma viscosity was calculated by multiplying the time for plasma displacement through the capillary by the instrument calibration factor.

Statistical analysis. For evaluation of statistical significance of the received results and differences revealed between used experimental conditions (changes in blood rheological properties, mistakes in maze tests and others) a one-way and two-way analysis of variance (ANOVA) were used.

temperature in the brain tissue does not change and is maintained approximately in the 36-36.5^{0}C interval, although the further increase of in HC temperature leads to increase of brain temperature, and when it reaches the level about 41^{0}C – the animal dies. Thus, in case of 38, 39 and 40^{0}C exposure in HC the the brain temperature of the test animals does not change drastically even if WBH exposure lasts several hours. In the next series of experiments we tested the animals learning ability in multi-branch maze after 2 and 4 hours

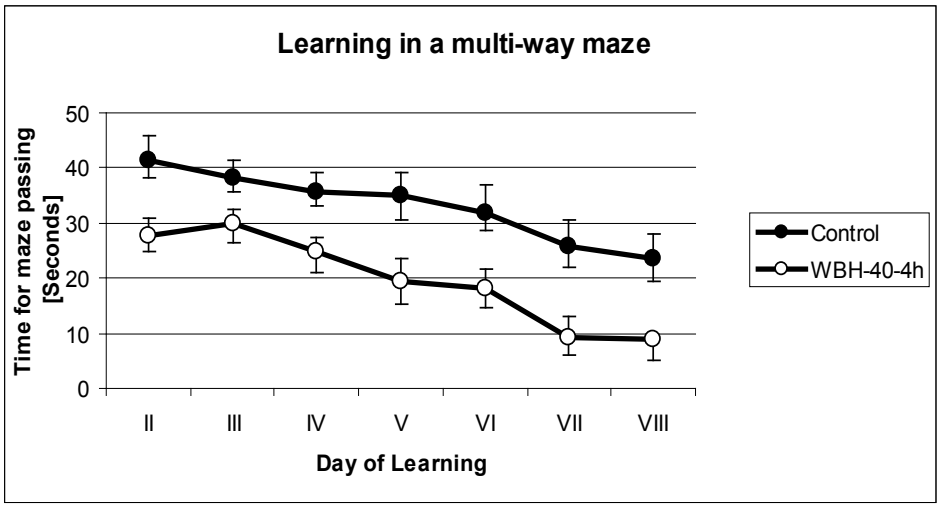

Fig. 1. Time of maze passing for the control and WBH exposed (4 hours, 40^{0}C) groups of test animals.

Results

Completion of the first stage of this study allowed us to get reliable information concerning the effects of WBH on the learning process in the multi-branch maze after temperature exposures at three different temperatures in a special hyperthermia chamber. Before analyzing the results of farther testing it is useful to remind on several important facts concerning the changes of brain temperature versus the changes of temperature inside the hyperthermia chamber (HC), namely, the phenomenon of autoregulation of brain temperature. The point is that if we increase the temperature into the HC up to 44-45^{0}C the hyperthermal exposure at the temperatures 38, 39 and 40^{0}C. The most pronounced results we received in case of 4 hours exposure by 40^{0}C: the total time of maze passing (in comparison with control animals (Fig. 1) and with those exposed at 38 and 39^{0}C) has been significantly shortened.

Interesting results have been received after determination of blood rheological properties in aforementioned experimental conditions. We found that the index of RBC aggregability is a significantly temperature-dependent parameter. Its changes during 4 hours WBH exposure at the different temperatures (example for 38^{0}C is presented on Fig. 2).

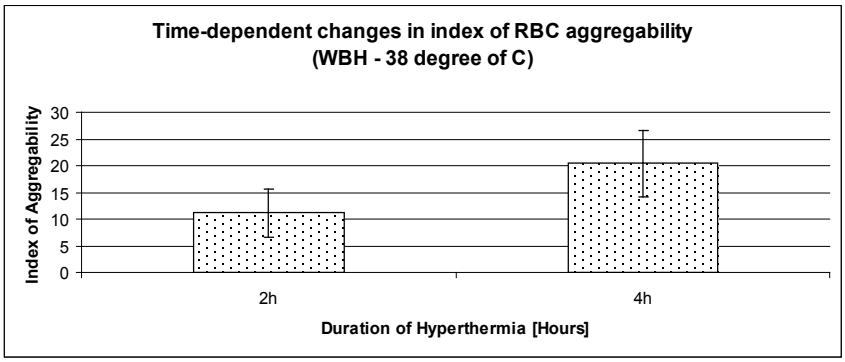

Fig. 2. Time-dependent changes in index of RBC aggregability.

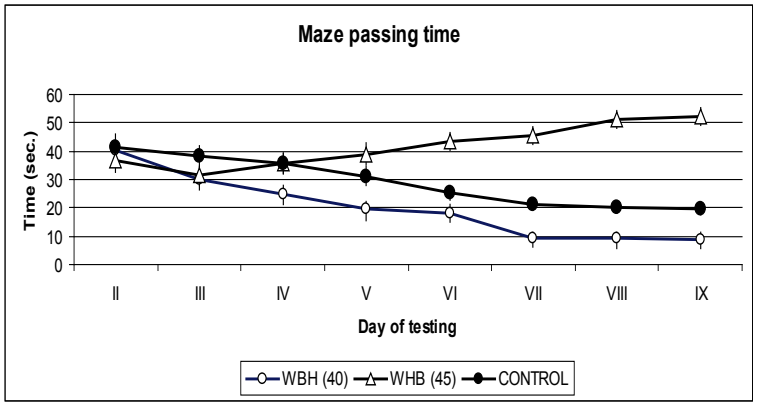

Fig. 3. Time of maze passing for control and WBH exposed (40 and 45^0C) groups of tested animals.

We have to underline that regardless of the duration of exposure we did not observe any statistically significant changes in the level of RBC deformability index caused by WBH exposure (38, 39 and 40^0C). The same should to be said concerning the changes in memory processes after WBH. The animals that reached the level of "automatic" behavior in the maze do not change this kind of behavior after WBH (38, 39 or 40^0C) exposure. As mentioned above, the most pronounced results were obtained after a 4 hours exposure at 40^0C (Fig. 1). The time of maze passing (in comparison with control animals and with those exposed to 38 and 39^0C) has been significantly shortened and we consider this fact as a well pronounced behavioral manifestation of WBH Hormetic effect. A highly significant increase in behavioral activity aimed at getting rid of from non-ethological conditions in response to oxidative stress (caused by hyper-thermal exposure), in our opinion, indicates that in this case the dose of induced stress was within the range needed for stimulation of Hormetic mechanisms. Taking into account that the most noticeable changes in animal's behavior were observed after exposure to temperature 40^0C we concentrate our attention on this temperature, but the obtained results encouraged us to use also 45^0C setting. Fig. 3 shows the effect of increased temperature (up to 45^0C) in the Hyperthermia Chamber on the behavior of the tested groups of animals.

As we see, beginning from the third day of testing, behavior of this group of animals differed drastically and the animals exposed to 45^0C hyperthermia needed more than 50 seconds (5 times more than in case of 40^0C, and 2.6 times more in comparison with the control group) to pass the maze. All differences were statistically significant

($p<0.01$). So, since the third day of testing Hormetic effect of hyperthermia exposure at 45^0 was disrupted. We can see that the main idea of Hormesis (dose-depending effect of induced stress) was manifested very clearly. In the following series of experiments we tried to reveal the influence of immune protective mechanisms, but also as a practically harmless method for testing toxicity by using experimental animals in full accordance with the 4R (replace, reduce, refine, responsibility) principles of the welfare of test animals.

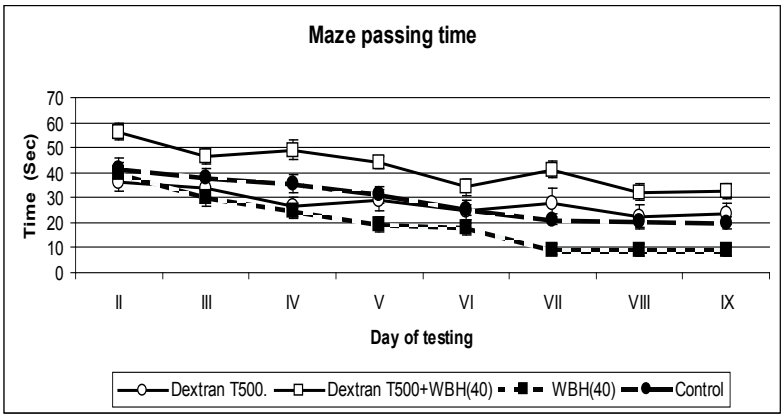

Fig. 4. Maze passing time of animals of Control, Dextran T500 injected, Whole Body Hyperthermia (40^0C) exposed groups and the group of animals received combination of WBH exposure and Dextran T500 injection.

artificially changed (aggravated) blood rheological properties caused by injection of 10% solution of high molecular Dextran T500 prior to maze experiments in combination and without WBH exposure (40^0C) on the animals behavior in four tested groups (4×12 animals). As shown on Fig. 4, behavior of control and Dextran injected groups does not differ significantly. The group of animals exposed to Hyperthermia (40^0C) manifested a pronounced Hormetic effect, while the same temperature exposure of animals with aggravated blood viscosity caused by injection of Dextran T500) significantly (and statistically proved) changed the animals locomotor activity and the phenomenon of Hormesis was eliminated. The experiments carried out also showed that all investigated effects have a fairly strong monotonic dependence on the liquid exposure dose in a wide range of measured parameters and their dimensionless combinations even at sufficiently low exposure doses very far from lethal and sub-lethal levels. Therefore, they can serve not only as an effective tool for stimulating of Hormesis and

Discussion

We have demonstrated that (under the studied conditions) the whole body hyperthermia facilitates in a temperature-dependent manner the learning processes and does not alter the memory processes. The phenomenon of brain temperature autoregulation has been revealed and the utilized whole body hyperthermia exposures up to 45^0C did not cause any changes in animal's brain temperature. Analysis of RBC aggregability revealed its exposure temperature dependent increase, while the RBC deformability remains practically at the normal level. From a biomedical point of view the term "Hormesis" describes phenomena, when in response to low doses of toxins or any other stressors, the body develops a positive reaction (from a biological standpoint) as an adaptive stress response, which can provide stability of cells to higher (fatal) doses of stressogenic factors stimulating the response. In recent years the interest to the Hormesis phenomenon has significantly increased and numerous attempts to investigate the physical,

chemical and psychological mechanisms (e. g. of the radiation Hormesis – protective effect of the low doses radioactive exposure) have been made. The resistance of living organisms to stresses is one of the most important indicators of its viability and it is clear that the study of the mechanisms that shape this resistance have a fundamentally important character [6]. So, the oxidative stress (a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of balance between them" [7]) is involved in the development of many pathological processes, but it may also play a significant role in the processes of physiological adaptation and regulation of intracellular signal transduction. According to the theory of D. Harman [8, 9], the oxidative stress plays a significant role in the processes of aging. Further development of this theory was given in the works, which argue that free radicals may contribute significantly to metabolic health and life expectancy [10]. This effect is known as Mitochondrial Hormesis (mitohormesis). Based on the all foregoing, the analysis of the obtained results allows us to conclude that in our experiments we observed behavioral manifestations of the Phenomenon of Hormesis. A very significant increase in behavioral activity aimed at getting rid of non ethological conditions in response to oxidative stress caused by hyperthermic exposure in our opinion indicates that in these cases the dose of induced stress was within the range needed for stimulation of Hormetic mechanisms. To make sure that we deal with Hormesis phenomenon we used the higher dose of hyperthermia exposure (45^0C). Finally, we observed the disruption of this phenomenon and acceleration of behavior was replaced by a well pronounced deceleration of animal's movement which is typical for the phenomenon of Hormesis, showing positive effect at low doses and negative effect at high doses of the stressor. Hyperthermal exposure can be an extremely effective anticancer modality in case of the local hyperthermia of tumor tissue because at the first stages it leads to increase of tissue oxygenation (which, according to the joint action of Warburg and Pasteur effects, is not a favorable condition for tumor cells) and on the next stages leads to an increased RBC aggregability and deterioration of blood viscosity causing a rapid slowing and even cessation of blood flow (as it was observed in case of 45^0C) in tumor tissue, disruption of glucose supply and killing of cells. In case of whole body Hyperthermia according to our results the tempera-ture should not exceed the 40^0C, when the level of increased RBC aggregability still allows to maintain blood flow in the whole organism on normal level (Hormetic effect is working) and all other benefits of hyperthermic exposure are still in action.

Conclusions

1. Stimulation of Hormetic mechanisms might be used not just for an adaptation to various kinds of stressors, but also for successful treatment of many pathological processes (including cancer); 2. The WHB at 40^0C might be used as one of the most effective triggering factors for launching of Hormetic mechanism, especially in cancer clinics, but it must not be used at higher or lower temperatures; 3. The localized hyperthermia of tumor tissues at 41-45^0C is a much more effective anticancer treatment modality than the WBH; 4. In all cases when we use Hormetic mechanism, both for adaptation or/and medical treatment, it is critical to make sure that the applied doses do not exceed the Hormetic range; 5. Fixation and interpretation of behavioral and blood rheology effects can be effectively used for toxicity testing of various synthetic and natural substances (including pharmaceuticals, BADs, antiviral and antimicrobial modalities, insecticides/acaricides/fungicides/herbicides, etc.) as a most advanced testing methodology in full accordance with the 4R (replace, reduce, refine, responsibility) principles of the welfare of test animals.

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ადამიანისა და ცხოველთა ფიზიოლოგია

მთელი სხეულის ჰიპერთერმიით გამოწვეული ჰორმეზისის ფენომენი (ექსპერიმენტული კვლევა)

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**საქართველოს ტექნიკური უნივერსიტეტი, თბილისი, საქართველო
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ჰიპერთერმია თითქმის ყოველთვის გამოიყენება კიბოს თერაპიის სხვა ფორმებთან ერთად, როგორიცაა ქირურგია, სხივური თერაპია და ქიმიოთერაპია. ჰიპერთერმიამ შეიძლება ზოგიერთი კიბოს უჯრედი უფრო მგრძნობიარე გახადოს დასხივების მიმართ, ან ზიანი მიაყენოს სხვა კიბოს უჯრედებს, რომლებზეც არ მოქმედებს რადიაცია. ნაკლებად ცნობილია მთელი სხეულის ჰიპერთერმიის (WBH) დასწავლისა და მეხსიერების პროცესებსა და სისხლის რეოლოგიურ თვისებებზე შესაძლო ზეგავლენის შესახებ. ამ საკითხების კვლევა წარმოადგენდა მოცემული ნაშრომის მთავარ საგანს, ხოლო კვლევის პროცესში წარმოიშვა ახალი სამიზნეები, რომელთა შესწავლამ გამოავლინა მთელი სხეულის ჰიპერთერმიის ახალი, მანამდე უცნობი სამკურნალო პოტენციალი. ლაბორატორიულ ვირთაგვებზე ჰიპერთერმიულ კამერაში ექსპოზირების პირობებში მრავალსვლიან ლაბირინთში ქცევით მაჩვენებლებზე დაკვირვების და სისხლის რეოლოგიური მახასიათებლების გაზომვის გამოყენებით ჩატარებულმა ექსპერიმენტებმა ცხადყო, რომ: 1. WBH შეიძლება გამოყენებულ იქნეს, როგორც ჰორმეზული ფენომენის წარმოქმნის და განვითარების ერთ-ერთი ეფექტური ფაქტორი. 2. ყველა შემთხვევაში, როდესაც ჩვენ ვიყენებთ WBH-ს, როგორც ჰორმეზული ფენომენის „გამშვებ ტრიგერს“, ონკოლოგიურ ან სხვა სახის კვლევებში პრინციპულად მნიშვნელოვანია, რომ ჰიპერთერმიის ტემპერატურული ინტერვალი იყოს „ჰორმეზული დიაპაზონის“ ფარგლებში. ეს უაღრესად მნიშვნელოვანია არა მხოლოდ ჰორმეზული მექანიზმის ეფექტუროზისათვის, არამედ სისხლის რეოლოგიური მაჩვენებლების შესაბამისი ნორმის ფარგლებში შესანარჩუნებლად.

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