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Effect of ultraviolet radiation on the free radicals formation in hypothyroid rat's liver

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Prolonged exposure to sunlight or solar simulated UV irradiation (SSUV) leads to oxidative stress in tissues. The literature data concerning the effect of this factor on hypothyroidism are controversial. The aim of this study was to investigate the influence of hypothyroidism and UV radiation on free radicals formation in rat's liver. After one week of adaptation, hypothyroid model was developed in 4 weeks, by continuous administration of 0.01% 6-n-propyl-2-thiouracil in the drinking water of male Wistar-Albino rats. Hypothyroidism was confirmed by the significant reduction of blood free thyroxin (approximately 0.44 ng/l, while 18 ng/l in the controls). The model was also proved by the loss of both appetite and body weight gain of the hypothyroid rats. During the 6-th week of the experiment, half of the PTU treated rats were irradiated with SSUV lamp for 60 min, divided into 4 portions with respective 15 min breaks. After decapitation, the accumulation of free radicals in rats' livers was measured spectrophotometrically using MTT-assay. Data were presented as percentage of the corresponding data for controls.

Alone, SSUV irradiation increased, while hypothyroidism decreased the free radicals accumulation in the rat liver. This was in agreement with the literature data about the individual effects of these factors on the oxidative stress. When the SSUV irradiation was applied on the hypothyroid rats, the relative increase of the free radicals in the liver was much higher than that in the livers of SSUV irradiated normothyroid animals.

Key words: hypothyroidism, UV radiation, free radicals, liver

INTRODUCTION

Prolonged exposure to sun or solar simulated UV (SSUV) irradiation leads to accumulation of free radicals in the skin [1], immune suppression and synthesis of excessive proinflammatory cytokines, all resulting in oxidative stress in different tissues [2,3]. The sunburn and UV-radiation are major factors for set-up and development of UV-initiated diseases [4-7]. Clinical, biochemical and histological observations showed that both humans and animals develop hypothyroidism during long lasting spaceflight [8]. This has been associated with effects of sub-apoptotic doses of UVC [9], which compromised thyrocytes proliferation and the expression of genes involved in thyroid hormones production.

Thyroid hormones are involved in setting of the basal metabolic rates in the liver [10] and in decreasing of the oxidative stress-induced toxicity both in animals and humans [11,12]. But literature data about oxidative stress levels in hypothyroidism are controversial. Thyroid dysfunction was associated with enhanced oxidative stress [13] due to reduced antioxidant defense [14,15] and increased free radicals production [16,17], although the interrelation is complicated. The oxidative stress in subclinical hypothyroidism has been associated with secondary hypercholesterolemia to thyroid dysfunction, but not to the hypothyroidism *per se* [18]. Other experimental data proved that the decreased metabolic rates due to hypothyroidism can diminish the tissue damages, opposing the deleterious effects of both increased free radicals and incapacitated antioxidant defense [19-21].

As thyroid hormones are involved in the control over the oxidative stress in a very complex manner, and the pre-exposition to solar (or solar simulated) UV radiation tends to initiate oxidative stress in tissues, the question arises about the effect of the prolonged exposition to sun or SSUV radiation on individuals with hypothyroidism.

In the present work, the effect of systemic pre-exposition to SSUV radiation on the free radicals production in the liver of hypothyroid rat model was estimated. The aim of the study was to monitor the individual and mutual effects of SSUV and hypothyroidism on the free radicals accumulation in the liver, and to compare this parameter with the corresponding level for the control animals. The accumulation of free radicals in a tissue is among the major factors for developing of the oxidative stress, later resulting in tissue damage. The free radicals accumulation was monitored by using

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spectrophotometric method, with MTT as a marker molecule. The activity of xanthine oxidase was estimated using uric acid as a marker.

MATERIALS AND METHODS

Animal model: 30 male Wistar albino rats of body weight 135±5 g were separated in 4 groups named C (control), UV (normothyroid rats exposed to SSUV), PTU (hypothyroid rats) and PTU+UV (hypothyroid rats exposed to SSUV), housed in transparent standard containers. All animals were treated in agreement with the General regulations for treatment of experimental animals, established by the Ethic Committee at the Medical University of Sofia, in agreement with the "Guide to the care and use of Experimental Animal Care" (Canadian Council on Animal Care Guidelines, 1984).

After one week of adaptation, the groups PTU and PTU+UV were provided with 0.01% (w/w) aqueous solution of 6-n-propyl-2-thiouracil (Sigma-Aldrich), *ad libitum* for 5 weeks. The water and food consumptions were measured everyday, at the same hour, and data were used to calculate the average daily food and water consumption of one animal of a group. The body weight of the rats was measured two times per week, and the weekly body weight gain of an animal in a group was estimated. The average daily dose of 6-n-propyl-2-thiouracil consumed by the model animals was 16±3 mg/kg_{BW}. At the end of the fourth week of the experiment, FT_4 was measured for each group. During the 5-th week, the normothyroid UV group and the hypothyroid PTU+UV group were exposed to ultraviolet radiation, by using UV lamp (type "Helios" 125W, IBORA, Bulgaria). The lamp combined UV (180 – 400 nm) and IR sources adjusted to mimic sunlight. The SSUV source was positioned at a distance of one meter from the animals' cage. The two groups were irradiated for 15 min four times per day, with periods of 15 min pause between sessions.

Preparation of the supernatanta: After the 7-th day of SSUV-exposure all animals were decapitated under anesthesia (Urethane, 2 mg/100 g BW). Livers were extracted and homogenized in sonified ice-cold PBS (50 mM, pH 7.45) solution of 0.04% BHT (for preventing the autooxidation with oxygen in the air). The homogenates were prepared using "Mechanik Prezsizna" type 302 homogenizer, at a speed of 2500 rpm and 20 vertical movements of the vessel. After centrifugation at 4°C and 2500 rpm for 10 min in a centrifuge (JERNETZKI K24), the supernatanta was collected and stored in ice-cold bath.

The amount of proteins in the supernatanta was determined as described by Stoscheck [22].

Xanthine oxidase activity assessment: The activity of xanthine oxidase was determined by measuring the relative change of the absorbance at 293 nm due to transformation of xanthine to uric acid, in a quartz cuvette, as previously described [23]. Briefly, one milliliter of the cuvette contained 0.02 ml xanthine solution, 0.02 ml supernatanta, and 0.96 ml PBS, against reference cuvette containing PBS. The blank measurement was performed by estimation of the relative change of the absorbance at 293 nm in a sample in PBS alone, with reference cuvette containing PBS. The amount of uric acid formed in the cuvette for one minute was calculated after subtracting the relative change of the absorbance at 293 nm measured in the blank sample. The activity of xanthine oxidase was calculated in mU/mg proteins, one unit of the enzyme being the amount needed to convert 1 μmole of xanthine to uric acid for one minute at 25°C. To assess the effect of the treatment on the xanthine oxidase activity, the latter was presented as a percentage of this for the control group.

Measurement of the free radicals accumulation: The accumulation of free radicals in the liver supernatanta was evaluated using a marker molecule named MTT (Nitroblue tetrazolium bromide; Sigma-Aldrich) [23,24]. In presence of free radicals MTT transforms to formazan [25], with characteristic absorbance at 578 nm [25,26]. Recently, MTT has been successfully used in evaluations of free radicals accumulation in presence of pharmaceuticals [24,27], plant extracts [28,29] and animal tissues [30], proving to be very efficient and cheap. One ml of the cuvette contained 0.02 ml liver supernatanta, 0.02 ml xanthine, 0.1 ml MTT and PBS. The relative change of the absorbance at 576 nm was monitored for 5 min. The amount of MTT formazan formed for one minute in the presence of supernatanta, containing 1 mg proteins was calculated, and then data were presented as percentage of these for the control animals.

Statistical analysis. All parameters were presented as percentages of the corresponding parameter for the control animals. The activity of the xanthine oxidase and the formation of MTT-formazan were treated as two factors, each of them having four levels ("control", "hypothyroid", "SSUV- irradiated", and "hypothyroid and SSUV-irradiated").

The statistical significance of the mean values and standard deviations for each factor were analyzed using Bartlett test, followed by ANOVA and Bonferoni post-test.

RESULTS

The hypothyroidism was achieved at the end of the 4-th week, as proved by both low free thyroxin (0.44±0.31 ng/l compared with 18.41±0.28 ng/l for the controls), loss of appetite, as well as by the loss of weight gain ($p<0.01$, Figure 1).

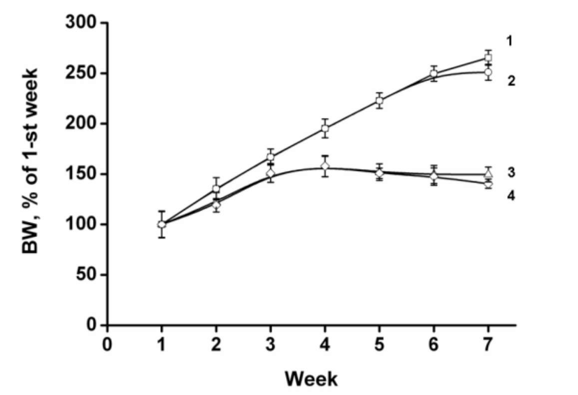

Fig. 1. Effect of 6-n-propyl-2-thiouracil on the body weight gain (BW, % of the 1-st week) of the experimental animals: 1 (□)- body weight of the control norm thyroid animals (group C); 2 (○)- body weight of the normothyroid rats exposed to SSUV-radiation (group UV); 3 (Δ)- body weight of the hypothyroid animals (group PTU); 4 (◇)- body weight of the hypothyroid animals exposed to SSUV-radiation for one week (group PTU+UV).

In agreement with literature [31-33], the loss of the body weight gain was associated with the decreased appetite of the animals, due to drastically decreased thyroid hormones levels.

Our data, presented in Figure 1, suggested that the hypothyroidism was the main factor for the loss of body weight gain ($p<0.001$). The SSUV exposure resulted in a slight but statistically significant ($p<0.05$) additional body weight loss for the normothyroid group.

When applied alone, SSUV treatment increased, while 6-n-propyl-2-thiouracil decreased the activity of xanthine oxidase, compared with the control group (Figure 2).

The activity of xanthine oxidase in the livers of the hypothyroid rats (group PTU) was (47±4)% of this in the livers of the control animals. This decrease could be related with the decreased overall metabolic rates of the animals due to 6-n-propyl-2-thiouracil-induced hypothyroidism [18, 20,21].

After one week of SSUV irradiation, the xanthine oxidase activity in the liver relatively increased to (115±1)% and (170±2)% for normothyroid and hypothyroid rats, respectively, compared to this in the livers of the corresponding untreated groups. In accordance with previously published data, this increased activity can be explained with adaptation-related oxidative stress [2,3].

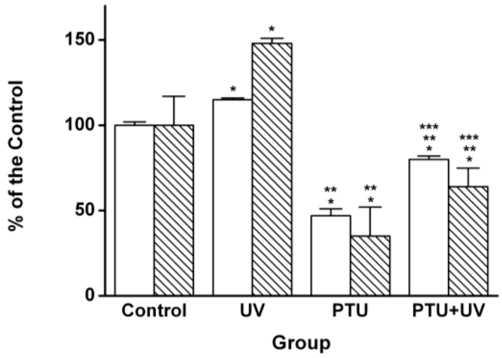

Fig. 2. Effects of SSUV-radiation and hypothyroidism alone and in combination on the activity of xanthine oxidase (□) and on the production of MTT- formazan (▨) in the liver homogenate of the model animals: Control – control group, PTU- hypothyroid rats, UV- normothyroid rats exposed to SSUV-radiation for 1 week, PTU+UV- hypothyroid rats irradiated for 1 week with SSUV. Data are presented as percentages of the corresponding parameters for the control group (* - $p<0.05$, **- $p<0.01$, *** - $p<0.001$).

However, we found, that even after SSUV exposure, the activity of xanthine oxidase in the livers of the group PTU+UV was still significantly lower than this of the control group. Statistical analysis suggested that in the hypothyroid-induced suppressed metabolism, the effect of the SSUV induced adaptive oxidative stress ($p<0.001$) due to the domination of xanthine oxidase activity in the rat liver.

The formation of MTT-formazan in the liver was enhanced due to SSUV irradiation to (148±3) %. In the hypothyroid state, the MTT-formazan decreased to (35±17) % in comparison with control animals. The SSUV exposure resulted in relatively more MTT-formazan in livers of normothyroid (148±3) % and hypothyroid (182±11) % rats, compared with the corresponding SSUV- untreated groups. The statistically significant ($p<0.001$) collective effect of SSUV and hypothyroidism on the MTT-formazan in the rat livers (64±11) %, compared to this of the control group (100±17) % indicated the prevailing impact of the SSUV.

DISCUSSION

As the MTT-formazan was formed by interaction of MTT with free radicals [23-30], its appearance in presence of our model systems indicated free radicals formation. In our investigation, the formation of free radicals in the liver supernatanta was prompted by addition of xanthine to the system. The increased content of xanthine provided enough substrate for xanthine oxidase to produce uric acid and reactive oxygen species [34,35].

Our study proved that, if applied alone, the 6-n-propyl-2-thiouracil-induced hypothyroidism decreased, while the SSUV-radiation increased the xanthine oxidase activity and free radicals liver accumulation. The former effect was associated with the decreased metabolic rates in the hypothyroid rats, while the latter was associated with adaptive UV-radiation induced oxidative stress.

The effects of the SSUV radiation on both xanthine oxidase activity and free radicals accumulation were stronger within the hypothyroid than within the normothyroid rats.

CONCLUSIONS

1. SSUV irradiation and hypothyroidism alone result in opposite effects on the oxidative stress in rat's liver: the former increases, while the latter decreases the free radicals accumulation in the liver tissue.
2. The SSUV treatment of hypothyroid rats resulted in less free radicals in their livers than these accumulated in the livers of normothyroid animals.
3. The relative increase of the oxidative stress in the hypothyroid rat's liver is higher than this in the normothyroid animal.

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REFERENCES:

1. F. A.Wagener, C. E. Carels, D. M. Lundvig, *Int. J. Mol. Sci.*, **14**, 9126 (2013).
2. H. Wei, X. Zhang, Y. Wang, M. *Cancer Lett.*, **185**, 21 (2002).
3. G. M. Halliday, D. L. Damian, S. Rana, S. N. Byrne, *J. Dermatol. Sci.*, **66**, 176 (2012).
4. R. K. Singh, M., Gutman, R. Reich, M., *Cancer Res.*, **55**, 3669 (1995).
5. A. Monnereau, S. L. Glaser, C. W. Schupp, K. Ekström Smedby, S. de Sanjosé, E. Kane, M. Melbye, L. Forétova, M. Maynadié, A. Staines, N. Becker, A. Nieters, P. Brennan, P. Boffetta, P. Cocco, I. Glimelius, J. Clavel, H. Hjalgrim, E. T. Chang, *Blood*, **122**, 3492 (2013).
6. P. Boffetta, O. van der Hel, A. Kricker, A. Nieters, S. de Sanjosé, M. Maynadié, P. L. Cocco, A. Staines, N. Becker, R. Font, A' Mannetje, C. Goumas, P. Brennan, *Int. J. Epidemiol.*, **37**, 1080 (2008).
7. T. Bald, T. Quast, J. Landsberg, M. Rogava, N. Glodde, D. Lopez-Ramos, J. Kohlmeyer, S. Riesenberg, D.-van den Boorn-Konijnenberg, C. Hömig-Hölzel, R. Reuten, B. Schadow, H. Weighardt, D. Wenzel, I. Helfrich, D. Schadendorf, W. Bloch, M. E. Bianchi, C. Lugassy, R. L. Barnhill, M. Koch, B. K. Fleischmann, I. Förster, W. Kastenmüller, W. Kolanus, M. Hölzel, E. Gaffal, T. Tüting, *Nature*, **507**, 109 (2014).
8. P. Dayanandan, *J. Biosci.*, **36**, 911 (2011).
9. E. Baldini, M. D'Armiento, S. Sorrenti, M. Del Sordo, R. Mocini, S. Morrone, L. Gnessi, F. Curcio, S. Ulisse, *Astrobiology*, **13**, 536 (2013).
10. A. Guererro, R. Pamplona, M. Postero-Olin, J. Barja, M. Lopez-Torrez, *Free Rad. Biol. Med.*, **26**, 73 (1999).
11. R. Gredilla, G. Barja, M. López-Torres, *Free Rad. Res.*, **35,** 417 (2001).
12. N. Petrovic, G. Cvijic, J. Djordjevic, V. Davidovic, *Ann. N.-Y. Acad. Sci.*, **1040**, 431 (2005).
13. U. Resch, G. Hersel, F. Tatzber, H. Sinzinger, *Clin. Chem. Lab. Med.*, **40**, 1132 (2002).
14. A. N. Torun, S. Kulaksizoglu, M. Kulaksizoglu, B. Q. Pamuk, E. Isbilen, N. B. Tutunku, *Clin. Endocrinol.* (*Oxf*), **70**, 469 (2009).
15. P. Pasupathi, R. Latha *Thyroid Sci.*, **3**, CLS1-6 (2008).
16. S. Mukherjee, L. Samanta, A. Roy, S. Bhanja, G. B. Chainy, *Biomed Res. Int*., Article ID 590897 (2014).
17. S. Yilmaz, S. Ozan S, F. Benzer, H. Canatan, *Cell Biochem. Funct.*, **21**, 325 (2003).
18. A. Santi, M. M. Duarte, C. C. Menezes, V. L. Loro, *Int. J. Endocrinol.*, Article ID 856359 (2012).
19. P. Venditti, M. Balestrieri, S. Di Meo, T. De Leo, *J. Endocrinol.*, **155**, 151 (1997).
20. M. J. Coria, A. I. Pastrán, M. S. Gimenez, *Acta Biomed.*, **80**, 135 (2009).
21. M. Messarah, A. Boumendjel, A. Chouabia, F. Klibet, C. Abdennour, M. S. Boulakoud, A. E. Feki, *Exp. Toxicol. Pathol.*, **62**, 301 (2010).
22. C. M. Stoscheck, *Methods Enzymol.*, **182**, 50 (1990).
23. B. Memedi, M. Traykova, N. Boyadjieva, *Trakia J. Sc*i., **10**, 321 (2012).
24. I. Kostova, M. Traykova, V. K. Rastogi, *Med. Chem.*, **4,** 371 (2008).
25. M.V. Berridge, P. M. Herst, A.S. Tan, *Biotechnol. Annu. Rev.*, **11**, 127 (2005).
26. R. H. Burdon, V. Gill, C. Rice-Evans, *Free Radic. Res. Commun.*, **18**, 369 (1993).
27. I. Kostova, M. Traykova, *Med. Chem.*, *2, 463 (2006).*
28. I. A. Muraina, M. M. Suleiman, J. N. Eloff, *Phytomedicine*, **16**, 665 (2009).
29. Y. Liu, M. G. Nair, *J. Nat. Prod.*, **73**, 1193 (2010).
30. M. Traykova, L. Astasidi, T. Traykov, N. Boyadjieva, *Trakia J. Sci.*, **10**, 336 (2012).
31. V. Welch-White, N. Dawkins, T. Graham, R. Pace, *Lipids Health Dis.*, **12,** 100 (2013).
32. S. Walrand, K. R. Short, L. A. Heemstra, C. M. Novak, J. A. Levine, J. M. Coenen-Schimke, K. S. Nair, *FASEB J.*, **28**, 1499 (2014).
33. R. Mullur, Y. Y. Liu, G. A. Brent, *Physiol. Rev.*, **94**, 355 (2014).

34. Y. Y. Sautin, R. J. Johnson, *Nucleosides Nucleotides Nucleic Acid*, **27**, 608 (2008).

35. G. Deliconstantinos, V. Villiotou, J. C. Stavrides, *Biochem. Pharmacol., 51, 1727 (1996).*

ЕФЕКТ НА УЛТРАВИОЛЕТОВОТО ОБЛЮЧВАНЕ ВЪРХУ ОБРАЗУВАНЕТО НА СВОБОДНИ РАДИКАЛИ В ЧЕРЕН ДРОБ НА ПЛЪХОВЕ С ХИПОТИРЕОИДИЗЪМ

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(Резюме)

Продължителното облъчване на здрави тъкани със симулирана слънчева ултравиолетова радиация (ССУВР) води окислителен стрес в тях. Литературните данни за ефекта на този фактор при хипотиреоидизъм са противоречиви. Цел на това изследване бе да се проучи влиянието на хипотиреоидизма и УВ радиацията върху образуването на свободни радикали в черен дроб на плъх. След едноседмична адаптация на мъжки бели плъхове от линията Вистар, хипотиреоидизмът у тях беше постигнат чрез хронично администриране на 0.01% 6-n-пропил-2-тиоурацил (пропицил) в питейната им вода за 4 седмици. Хипотиреоидизмът бе потвърден от значителното намаление на тироксина в кръвта на моделните животни (около 0.44 нг/л, при 18 нг/л за нормотиреоидните плъхове). Хипотиреоидизмът бе потвърден и от загубата на апетит, и от забавеното относително нарастване на телесното тегло на моделните животни.

През шестата седмица на опита, половината от третираните с пропицил плъхове бяха облъчвани с лампа, симулираща слънчева УВ радиация в продължение на 60 минути, групирани в 4 равни интервала, с междинни прекъсвания от по 15 минути.

След декапитиране на опитните животни, натрупването на свободни радикали в черния дроб бе изследвано спектрофотометрично, с прилагането на МТТ-методика. Резултатите за моделната група бяха представени като процент от съответните данни за контролната група.

Приложени поотделно, ССУВР увеличаваше, докато хипотиреоидизмът намаляваше натрупването на свободни радикали в черен дроб на плъх. Този резултат бе в съгласие с литературните данни за индивидуалните ефекти на тези фактори върху окислителния стрес. След прилагане на ССУВР върху хипотиреоидни плъхове, натрупването на свободни радикали в черния им дроб беше много по-голямо от това в черния дроб на третирани по същия начин нормотиреоидни животни.