

Exhausted antioxidant defense in SSUV-exposed skin of hypothyroid rats

G. S. Bocheva^{1*}, T.E. Yordanova², M. L. Valcheva-Traykova², T. Traykov²

¹Department of Pharmacology and Toxicology, Faculty of Medicine, Sofia Medical University, 2 Zdrave Str., 1431 Sofia, Bulgaria

²Department of Medical Physics and Biophysics, Faculty of Medicine, Sofia Medical University, 2 Zdrave Str., 1431 Sofia, Bulgaria

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The decrease of the overall antioxidant defense due to solar simulated ultraviolet irradiation or hypothyroidism has been reported both in humans and animal models. Using a rat model, we aimed to investigate how the combination of UV radiation and hypothyroidism affects the antioxidant defense in the photo-exposed skin. The antioxidant resistance of the rat skin was characterized directly by the radical scavenging (RSA) activity toward stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH^{*}), and indirectly by the formation of free radicals due to Fenton reaction-induced oxidative stress. Four groups of male Wistar albino rats, named C (controls), SSUV (irradiated), PTU (hypothyroid), and PTU+SSUV, were used in this experiment. Drug-induced hypothyroidism was developed by the addition of 0.01% (w/w) 6-n-propyl-2-thiouracil (PTU) for 5 weeks in the *ad libitum* consumed drinking water. Then SSUV and PTU+SSUV groups were irradiated for 7 days. The results showed drastically lower antioxidant activity of the H-donors in PTU+SSUV skin than that in the skin of healthy controls. In PTU and SSUV groups a lower antioxidant activity than controls was found as well, the decrease being in the order: C > PTU ≥ SSUV >> (PTU+SSUV). Free radical accumulation was many times higher in SSUV-treated euthyroid skin compared to non-irradiated skin of controls. The Fenton reaction in the PTU group resulted in the formation of very few free radicals in the skin that might be related with the better RSA and slowed the metabolism of the hypothyroid rats. In conclusion, the combination of chronic sun exposure and hypothyroidism could be a risky and harmful factor leading to exhausted antioxidant defense and possible skin damage.

Keywords: Hypothyroidism, UV radiation, SSUV, Antioxidant defense, Skin, DPPH^{*}

INTRODUCTION

Skin - the external body organ is chronically exposed to sunlight, where the ultraviolet (UV) radiation is one of the most harmful exogenous factors with negative biological effects [1]. UV radiation is a well-recognized generator of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which play a key role in mediating its biological effects. Under the control of endogenous antioxidants, these species participate in redox-dependent regulation of cell metabolism in response to UV stress, but if unbalanced, they induce oxidative damage. Their accumulation is considered as a risk factor in photoaging, photoimmunosuppression and photocarcinogenesis.

Thyroid hormones have a strong impact on oxidative status of the body [2]. Long lasting hypothyroidism substantially affects multiple organs and systems [3-5], leading to increased level of oxidative stress (OS) [3,6-8]. Recently, it was observed that the mean basal serum total antioxidant status (TAS) was lower, while serum total oxidant status (TOS) and OS index were significantly higher in the blood of hypothyroid patients. TOS has been positively correlated with free levothyroxine (fT4) and negatively correlated with the thyroid

stimulating hormone (TSH) [9]. Moreover, hypothyroidism was found to decrease the antioxidant enzyme activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, and reduced glutathione [10]. Thyroxine application, alone or in combination with apelin, demonstrated a beneficial effect of lowering lipid peroxidation and increasing antioxidant enzyme activity in humans and rodents [11,12].

The decrease of the overall antioxidant defense due to solar simulated ultraviolet (SSUV) exposure [13-16] or hypothyroidism [17-19] has been reported in both humans and animal models.

No data have been published so far on the mutual effects of the overt hypothyroidism and prolonged sunlight exposure on antioxidant defense of the skin. Therefore, using animal model, we aimed to investigate how the UV radiation together with hypothyroidism affect the antioxidant defense in the photo-exposed rat skin.

The antioxidant resistance of the rat skin was characterized directly by the radical scavenging activity (RSA) toward stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH^{*}), and indirectly by the formation of free radicals due to Fenton reaction-induced oxidative stress.

* To whom all correspondence should be sent.

E-mail: bocheva_georgeta@yahoo.com

All chemicals used in this study were of the highest grade available (Sigma-Aldrich).

Animal model

36 male Wistar albino rats of body weight (BW) 135 ± 5 g were assigned to 4 groups: C (control), SSUV [euthyroid rats exposed to solar simulated ultraviolet (SSUV) radiation], PTU (hypothyroid rats) and PTU+SSUV (SSUV treated hypothyroid rats), all housed in transparent standard containers. The animals were kept at room temperature (25 ± 0.5 °C), standard humidity (60 ± 1 %) and a light/dark (12/12 h) cycle. All animals were treated in agreement with the general regulations for treatment of experimental animals, established by the Ethics Committee of the Medical University of Sofia, in agreement with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

After one week of adaptation, hypothyroidism was induced in the PTU and PTU+SSUV groups by administration of 0.01% (w/w) 6-n-propyl-2-thiouracil (PTU) for five weeks in the *ad libitum* consumed drinking water. The BW of the rats was measured on a daily basis. Average weekly BW gain and average daily BW of each animal for the week were calculated for every group. The average daily dose of PTU consumed by the model animals was determined on the basis of the average daily consumption of PTU solution, and was found to be 16 ± 3 mg/kg BW. At the end of the fourth week of the experiment, thyroid hormones were measured for each group. During the final week, euthyroid (SSUV group) and hypothyroid rats (PTU+SSUV group) received ultraviolet radiation, using a SSUV lamp (type "Helios", UV-125W/IR-175W, IBORA, Bulgaria). The lamp combined UV (180 – 400 nm) and IR sources that were 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 for seven days with periods of 15 min rest between sessions (UV-45 mJ/sm²; IR-63 mJ/sm²). Our UV irradiation model was modified from Erden Inal *et al.* [20] to avoid radiation-inflicted burns and to mimic low-dose daily sunlight.

After the seventh day of SSUV- exposure, skin samples were taken from half of the animals in each group and used to analyze the antioxidant potential of the skin.

Preparation of the supernatant

Skin tissue was stored and homogenized in a sonified ice-cold PBS (50 mM, pH 7.45) solution of 0.04% 3,5-di-*tert*-4-butylhydroxytoluene (BHT) (to

prevent autoxidation) [21-23]. After centrifugation at 4°C and 7500 rpm for 15 min, the supernatant was collected and stored in an ice-cold bath. The amount of proteins (in mg/ml) in the supernatant was determined as described by Stoscheck [24].

Assay for RSA toward DPPH•

The relative decrease in absorption of the signal at 517 nm (characteristic band for DPPH•) was monitored for 10 min using the kinetic software of the apparatus. The absorption at 517 nm was recorded every minute. RSA (%) was determined using the formula:

$$RSA = \frac{A_{blank} - (A_{sam.} - A_{contr.})}{A_{blank}} * 100,$$

A_{blank} being the absorption due to the presence of the sample's solvent in the DPPH• solution (2 ml DPPH• solution and 0.02 ml PBS), $A_{contr.}$ is the absorption due to the sample alone (0.02 ml sample solution in 2 ml ethanol), and A_{sam} is the absorption due to interaction of the sample with DPPH• (2 ml DPPH• solution and 0.02 ml sample in PBS). RSA is presented as a percentage of the value obtained for the control group.

Fe(II)-induced free radical accumulation (FRA) assay

FRA in the supernatant was initiated by the Fe(II)/H₂O₂/EDTA/ascorbate model system in PBS medium, and the formation of MTT-formazan from MTT was used as a marker. The relative increase of the intensity at 578 nm (characteristic for MTT-formazan) was monitored each minute, for 10 min. FRA was evaluated using the formula:

$$FRA = \frac{\Delta A_{blank} - (\Delta A_{sam.} - \Delta A_{contr.})}{\Delta A_{blank}} * 100,$$

ΔA being the relative change of the absorption at 578 nm for 10 min. ΔA_{blank} corresponds to ΔA in the presence of the OH•-producing model system alone (0.05 ml Fe(II)/H₂O₂/EDTA, 0.05 ml ascorbate, 0.2 ml MTT, and PBS to 2 ml), $\Delta A_{contr.}$ describes the relative change of A (578 nm) in the presence of supernatant alone (0.2 ml MTT, supernatant containing 1 mg/ml proteins and PBS to 2.0 ml), and ΔA_{sam} shows the relative change of the 578 nm signal due to interaction between the model system and the supernatant (0.05 ml Fe(II)/H₂O₂/EDTA, 0.05 ml ascorbate, supernatant containing 1mg/ml proteins, 0.2 ml MTT, and PBS to 2 ml). Data are presented as a percentage of data for the controls.

Statistical analysis

The standard statistical software package was applied for statistical evaluation of data. The

significance in differences among standard deviations was verified using Bartlett test. One way ANOVA test was performed, followed by Bonferroni post-test.

RESULTS

Similar to our previous research [8], here we also found a negative impact of hypothyroidism on the

growth and weight gain, and slowed metabolism. The total antioxidant capacity of all hydrogen donors in the SSUV-treated skin of hypothyroid rats was determined using discoloration of DPPH[•] (517 nm), as presented in Fig. 1.

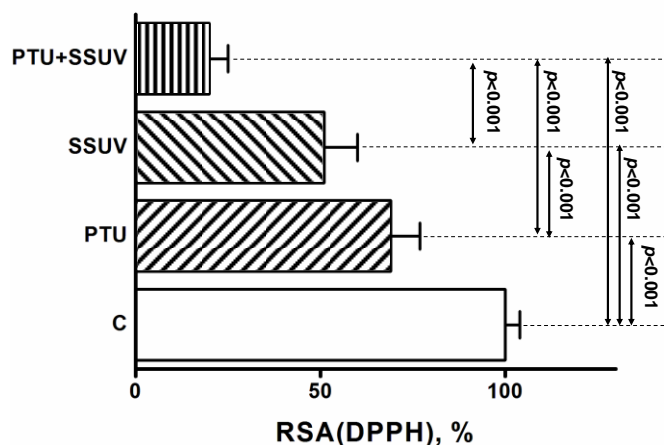


Fig. 1. Total antioxidant capacity toward DPPH[•]. Control group (C), SSUV-exposed group (SSUV); propylthiouracil-induced hypothyroid group (PTU), and propylthiouracil-induced hypothyroid group exposed to SSUV radiation (PTU+SSUV).

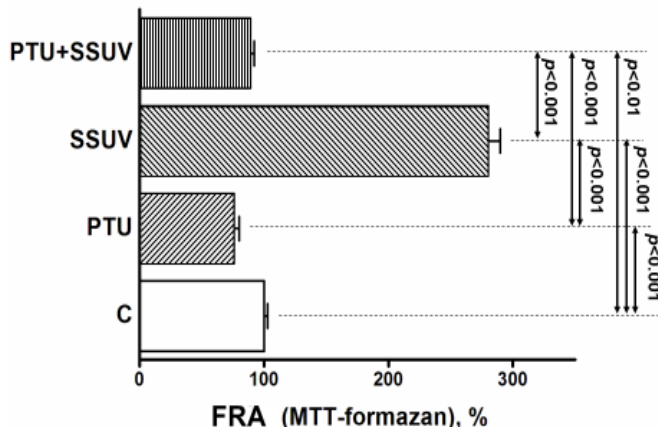


Fig. 2. FRA in a model system generating OH[•](Fe²⁺/H₂O₂/EDTA/ascorbate). Control group (C), SSUV-exposed group (SSUV); propylthiouracil-induced hypothyroid group (PTU), and propylthiouracil-induced hypothyroid group exposed to SSUV radiation (PTU+SSUV).

The results showed drastically lower antioxidant activity of the H-donors in PTU+SSUV rat skin than that in the skin of healthy controls. In PTU and SSUV groups a lower antioxidant activity than controls was found too, the decrease being in the order: $C > PTU \geq SSUV >> (PTU+SSUV)$.

To further monitor the antioxidant activity of photo-exposed hypothyroid rat skin, Fenton reaction

was initiated by Fe(II)/H₂O₂/EDTA/ ascorbate model system (Fig. 2). FRA was many times higher in SSUV-treated euthyroid skin compared to the non-irradiated skin of controls. Hypothyroid skin demonstrated a decrease ($p < 0.001$) and after irradiation a slight increase ($p < 0.01$) of FRA, both statistically significant, in comparison with FRA in healthy skin of controls.

DISCUSSION

The current study provided important data about the impact of the combination of chronic sun exposure and hypothyroidism on the antioxidant defense of the skin.

In addition to other research findings about decreased antioxidant defense measured in the blood of hypothyroid patients [9,10], we found a reduced antioxidant protection in the skin of our PTU-induced model. This approach allowed us to observe that SSUV exhausted more hydrogen (H) donating antioxidants than the hypothyroidism, while the combination of the two factors led to a very strong decrease of the RSA.

The Fenton reaction resulted in the formation of very few free radicals in the skin of PTU compared to the SSUV group. The lower FRA might be related with the better RSA and slowed metabolism of the PTU rats. These results are in agreement with other studies in the literature showing a decrease in specific and total oxidative capacity in many hypothyroid tissues with active metabolism, such as liver, heart, and brown adipose tissue [25-27]. Evidently, the metabolic rates play a decisive role in the accumulation of free radicals in SSUV-irradiated skin.

Antioxidants with radical scavenging properties are promising therapeutics against oxidative stress in hypothyroid patients being chronically exposed to UV radiation.

CONCLUSIONS

1. SSUV exposure of hypothyroid rats resulted in compromised RSA in the skin, compared to irradiated euthyroid rats.

2. Combination of chronic sun exposure and hypothyroidism could be a risky and harmful factor leading to exhausted antioxidant defense and possible skin damage.

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ИЗТОЩЕНА АНТИОКСИДАНТНА ЗАЩИТА В КОЖАТА НА ХИПОТИРОИДНИ ПЛЪХОВЕ СЛЕД ОБЛЪЧВАНЕ СЪС СИМУЛИРАНА СЛЪНЧЕВА СВЕТИЛИНА

Ж. Ст. Бочева*, Т. Е. Йорданова², М. Л. Вълчева-Трайкова², Т.Трайков²

¹Катедра по фармакология и токсикология, Медицински факултет, Медицински университет - София, ул. „Здраве“ 2, 1431 София, България

²Катедра по медицинска физика и биофизика, Медицински факултет, Медицински университет - София, ул. „Здраве“ 2, 1431 София, България

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(Резюме)

Намаляване на общата антиоксидантна защита след облъчване със слънчева светлина или при хипотиреоидизъм е съобщавано както при хора, така и при животински модели. Използвайки плъхове като модел, си поставихме за цел да изследваме как комбинацията от ултравиолетова радиация и хипотиреоидизъм действа върху антиоксидантната защита на фото-експозирана кожа. Антиоксидантната резистентност на кожата беше охарактеризирана директно чрез радикал-извличаща активност (RSA) към стабилен 2,2-diphenyl-1-picryl-hydrazyl радикал (DPPH^{*}) и индиректно чрез образуване на свободни радикали при индуциран оксидативен стрес в резултат на Fenton реакция. За експеримента бяха използвани 4 групи мъжки плъхове, порода Wistar albino, означени като: С (контрола), SSUV (облъчени), PTU (хипотиреоидни), и PTU+SSUV. Лекарствено-индуцираният хипотиреоидизъм беше получен чрез 0.01% 6-n-propyl-2-thiouracil (PTU) във водата им за пиене за период от 5 седмици. След този период, групите SSUV и PTU+SSUV бяха облъчени за 7 дни. Резултатите показаха драстично по-ниска антиоксидантна активност на донорите на водород в кожата на групата PTU+SSUV, отколкото в кожата на здравите контроли. При PTU и SSUV групите беше намерена също по-ниска антиоксидантна активност, в сравнение с контролата: $C > PTU \geq SSUV > (PTU + SSUV)$. Натрупването на свободни радикали беше многократно по-голямо при облъчената еутиреоидна кожа, в сравнение с необлъчената кожа на контролите. Реакцията на Fenton при PTU групата доведе до образуване на незначително количество свободни радикали в кожата, което би могло да се свърже с по-добрата RSA и забавения метаболизъм на хипотиреоидните плъхове. В заключение, комбинацията от хронично слънчево облъчване и хипотиреоидизъм би могла да бъде рисков и увреждащ фактор, водещ до изтощена антиоксидантна защита и възможно увреждане на кожата.