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Original Article

The effect of sunblock against oxidative stress in farmers: a pilot study

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Abstract

Farmers are frequently exposed to ultraviolet (UV) radiation which causes various diseases by inducing oxidative stress. This study aimed to assess the effects of sunblock on oxidative stress in the body. Eighty-seven farmers were divided into two groups: those who wore sunblock for five days and those who did not. The total antioxidant capacity (TAC) in urine, which is an antioxidant indicator, and 8-hydroxy-2-deoxyguanosine (8-OHdG) levels in urine, an oxidative stress indicator, were measured. The urinary TAC of sunblock users was significantly higher than that of non-users, but urinary 8-OHdG levels were not significantly different. Even after adjustment for potential confounders, urinary TAC was found to be markedly increased with sunblock usage. These results suggest that sunblock is effective in preventing oxidative stress among farmers. In addition, they show that urinary TAC can be used as a good effect marker of oxidative stress caused by UV exposure.

Keywords: farmers, ultraviolet rays, sunscreening agents, oxidative stress, total antioxidant capacity

Introduction

Exposure to ultraviolet (UV) radiation is an important risk factor in the development of various skin diseases such as skin cancer or erythema, and ocular exposure is the main cause of cataracts, macular degeneration, and malignant eyelid pathologies[1–3]. The primary source of UVA radiation (315–400 nm wave length) is sunlight. Solar radiation is an important occupational exposure to investigate since skin cancer incidence and mortality rates have been steadily increasing over the past decade. Moreover, this tendency is likely to increase further with ongoing depletion of the ozone layer. At last, in 1992, the International Agency for Research on Cancer classified solar radiation as a group 1 carcinogenic hazard, that is, known to cause cancer in people[4]. Although most of the UVA we are exposed to is absorbed by the dermal-epidermis, approximately 10% can penetrate as deep as the hypodermis. UVA has lower energy than UVB (280–315 nm wavelength), and it often produces indirect effects by generating reactive oxygen species (ROS), which then react with macromolecules like DNA or proteins to cause various diseases[5]. The transversion guanine to thymine is well known mutation caused by ROS by oxidizing guanine at the 8th position to produce 8-hydroxy-2-

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deoxyguanine (8-OHdG), which tends to pair with an adenine instead of cytosine. Such mutation can be found in tumors isolated from skin[6].

Farmers belong to an occupational group that gets more frequent exposure to the sun's UV rays, and they are known to have higher incidences of skin cancer and eye diseases than the general population[7–8]. In Australia, which has the highest rate of skin cancer in the world[9–10], farm workers have been shown to have the highest mortality rate due to melanoma and other skin cancers[11]. Taken together, it is clear that shielding agricultural workers from UV rays is crucial to preventing such diseases.

To reduce the risk of skin cancer, the American Academy of Dermatology and American Cancer Society recommend that individuals limit their time spent in the sun; avoid the sun's rays between 10 a.m. and 3 p.m.; use sunblock with an SPF of 15 or more; wear sun protective clothing like sunglasses, wide-brimmed hats, long sleeve shirts, and long pants; stay in the shade whenever possible; examine their skin regularly. Farmers, however, have difficulty adopting some of the recommended behaviors given the realm of their work[12]. In that sense, sunscreen can be for farmers to be the most realistic sun protection methods. In a recent study from Australia, aging of the skin occurred 24% more slowly in people who used sunblock every day than in those who did not use sunblock. In addition, not only does sunblock usage prevent sunburns and suntans, but it also protects against skin cancers such as squamous cell carcinoma[13]. These findings suggest that sunblock inhibits oxidative stress in the body.

On a different note, other study has reported that zinc oxide contained in sunblock actually causes oxidative stress on the skin during UV exposure; this causes aging of the skin, as well as damage to the skin cells and DNA[14]. Further research is needed on this matter. Hence, in order to determine the ability of sunblock to protect against UV radiation, we assessed total antioxidant capacity (TAC), as well as the 8-OHdG levels, in the urine of agricultural workers; these parameters are indicators of oxidative stress.

Materials and methods

Participants

The study participants included 86 healthy volunteers who are farming open field or greenhouse at Chungbuk province, Korea. Individuals were given information about the purposes of this study, and those who wished to participate provided written consent. Face-to-face interviews were conducted by experienced interviewers using a standard questionnaire that included demographic questions as well as questions on the history of smoking, alcohol consumption, and information related to farming work including sun protection equipment. The subjects were then split into two groups by self-selection: those who wore sunblock and those who did not. Individuals in sunblock using group wore sunblock before farm work for five consecutive days in July and August, when UV exposure is the greatest, and their spot urine was collected daily. The study protocol was approved by the institutional review board of Chungbuk National University Hospital (CBNUH-2015-06-019).

CUPRAC-BCS assay for total antioxidant capacity

To evaluate TAC, the copper (II) reduction assay with bathocuproinedisulfonic acid disodium salt (BCS) as the chelating agent (CUPRAC-BCS assay) was used as previously described[15]. Briefly, each urine sample of subjects was diluted 1:40 with 0.25 mmol/L BCS in phosphate buffer, pH 7.4. Then, 200 μL of each sample was put into each well of a 96-well plate, and the baseline absorbance was measured at 490 nm. After adding 50 μL of 0.5 mmol/L $CuSO_4$ solution, the reaction mixture was incubated for 3 minutes at room temperature. The reaction was stopped with 50 μL of 0.01 mol/L EDTA, and the final absorbance was measured at 490 nm using the microplate reader (Bio-Rad, Hercules, CA, USA). The difference between the final and the baseline absorbance was used to assess TAC.

Measurements of urinary 8-OHdG level

Urinary 8-OHdG levels were determined using a commercial enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Nikken SEIL, Shizuoka, Japan), according to the manufacturer's instruction. Briefly, 50 μL of urine samples and standards were added to precoated 8-OHdG protein conjugate microtiter plates, followed by 50 μL of the primary antibody, anti-8-OHdG monoclonal antibody solution, and incubated for 1 hour at 37°C. The plates were washed and the enzyme-labeled horseradish peroxidase-conjugated secondary antibody (100 μL) was applied for 1 hour at 37°C. After washing, 100 μL of the chromatic substrate, (3,3′,5,5′)-tetramethylbenzidene, was added to the plate and allowed to react at room temperature for 15 minutes. The intensity of color produced for each sample was measured at an optical density of 450 nm. The urinary 8-OHdG concentration (ng 8-OHdG/g creatinine) was adjusted to the urinary concentration of creatinine to control for the variability in urine dilution. The calibration range was 0.125-10 ng/mL, with a regression coefficient of 0.999. The

analytical precision of triplicate analyses was within 5%.

Statistical analyses

Statistical analyses were performed using SPSS version 21.0 (IBM, Armonk, NY, USA). Differences in demographic factors among groups were compared using the chi-square test. Statistical comparisons of means were performed using Student's *t*-test, and multiple linear regression analysis was applied to test relationships between variables. Statistical significance was set at $P < 0.05$.

Results

Table 1 shows the demographic characteristics of the study subjects. Among the study subjects, 48 chose sunblock using group, and 38 did not. There was no significant difference in mean age between sunblock using and non–using groups (66.89±10.94 vs. 68.34±11.32). Average daily working time was 3.21±1.32/day and 2.74±11.31/day in sunblock users and non-users, respectively ($P > 0.05$). Women accounted for 39 (83.0%) of sunblock users, which was significantly higher than the 16 (42.1%) of non-users who were women. In terms of the type of farm work, open field workers represented 34 (72.3%) of all sunblock users, and 37 (97.4%) of non-users; this was a statistically significant difference ($P < 0.01$).

Sunblock users were compared with non-users in terms of the proportions of subjects who wore short-pants and short-sleeved top (group 1), short-pants and long-sleeved top or long-pants and short sleeved top (group 2), and long-pants and long-sleeved top (group 3) during work. The results showed that a significantly higher proportion of sunblock users than of non-users wore long-sleeved clothing (78.7% *vs*. 34.2%, $P < 0.001$). The proportion of subjects who wore other forms of protection against sunlight (hats, sunglasses, *etc*.) besides sunblock was also significantly higher among sunblock users than among non-users (100% *vs*. 84.2%, $P < 0.01$).

The mean urinary TAC and 8-OHdG for 5 days were used to compare between groups after adjustment for age, gender, work type, smoking status, working hours, and usage of sunblock and other protection (***Table 2***). The mean urinary TAC in sunblock users was 0.49±0.16, while that in non-users was 0.36±0.17; this constituted a significant difference ($P = 0.022$). With regard to urinary 8-OHdG, sunblock users had somewhat lower levels than non-users (7.76±4.66 *vs.* 7.93±3.21), but there was no significant difference ($P = 0.947$).

Table 1 **Distribution of demographic factors in the study subjects**

	Sunblock users	Non-users	*P* value
N	48	38	
Age, years	66.89±10.94	68.34±11.32	0.552
Working time /day, hours	3.21±1.32	2.74±11.31	0.101
Gender, n(%)			<0.001
Male	8 (17.0)	22 (57.9)	
Female	39 (83.0)	16 (42.1)	
Smoking, n(%)			0.320
Smoker	1 (2.1)	3 (7.9)	
Non smoker	46 (97.9)	35 (92.1)	
Work type, n(%)			0.005
Open field	34 (72.3)	37 (97.4)	
Greenhouse	13 (27.7)	1 (2.6)	
Type of working clothes, n(%) [a]			<0.001
Group 1	1 (2.1)	6 (15.8)	
Group 2	9 (19.2)	19 (50.0)	
Group 3	37 (78.7)	13 (34.2)	
Using hat or sun glasses, n(%)			0.006
Yes	48 (100)	32 (84.2)	
No	0 (0.0)	6 (15.8)	

[a]Type of working clothes; Group 1: Short-pants and short-sleeved top, Group 2: Short-pants and long-sleeved top or long-pants and short sleeved top, Group 3: Long-pants and long-sleeved top

Table 2 **Urinary total antioxidant capacity and 8-OHdG levels among sunblock users and non-users**

	Sunblock users	Non-users	*P* value
Urinary TAC (mmol/L UA equiv./mmol/L creatinine) [a]	0.49±0.16	0.36±0.17	0.022
Urinary 8-OHdG (ng/g creatinine) [a]	7.76±4.66	7.93±3.21	0.947

[a]Adjusted for age, gender, type of work, smoking status, working time/day, use of other protective measures, and working clothes

A multiple regression analysis was conducted wherein the independent variables were gender, age, smoking status, average number of working hours during daylight, type of work, use of sunlight protection besides sunblock, and sunblock use; among these, sunblock use was the factor most strongly correlated with the urinary TAC (P = 0.002) (***Table 3***). Using sunblock was associated with an increase of 0.106 mmol/L UA equiv./mmol/L creatinine in TAC concentrations compare to non-users, after adjusting various factors. The analysis also showed that the TAC was reduced more in women who used sunblock than in men who did so, and that the TAC was lower among smokers, workers with longer work hours, and those who worked in a field rather than in a greenhouse. In addition, the TAC was found to be higher in those who wore long-sleeved clothing or other protection during work. However, none of these differences was significant.

Discussion

Many studies have been published addressing the ability of UV radiation to induce oxidative stress in the body. The ROS generated by UV radiation are known to cause DNA damage, such as DNA single-and-double strand breaks or base modifications; appropriate sunlight-blocking protection is critical for preserving health[16–17]. In the present study, the proportion of field workers who used sunblock was higher than that of greenhouse workers who did so, and the average number of hours worked under daylight was also higher among sunblock users. Nevertheless, the urinary TAC was significantly higher in farmers who used sunblock before work than in non-users. This is consistent with the results of other studies[13,18], and indicates that sunblock inhibits the oxidative stress caused by exposure to sunlight.

Not only does UV exposure increase the generation of ROS, many studies have reported that it also decreases the levels of various antioxidant substances[19]. In the present study, the urinary TAC was significantly lower in sunblock users than in non-users; this held true even after correction for many possible confounders in the multiple regression analysis. Therefore, sunblock use is a significant determinant of the urinary TAC. Smoking, as well as working long hours, reduced the TAC, while using other forms of protection, or wearing long-sleeved shirts, increased TAC; however, none of these differences was significant. This result may be caused by small sample size.

According to a study by Svobodová *et al.*[5], UVB radiation reduces the activity of antioxidant enzymes such as superoxide dismutase, glutathione S-transferase, chloramphenicol acetyltransferase, *etc.* In addition, a study by Vilela *et al.*[20] reported that UV radiation significantly decreases glutathione levels, but also that these depleted levels are restored following the use of

Table 3 **Result of multiple linear regression analysis for urinary TAC concentration**

Variables	Urinary TAC (mmol/L UA equiv./mmol/L creatinine)			
	β	SE (β)	T	*P* value
Age (years)	0.002	0.001	1.464	0.147
Gender (Men = 0, Women = 1)	–0.015	0.032	–0.486	0.629
Smoking status (No = 0, Yes = 1)	–0.047	0.068	–0.686	0.495
Working time/day (hour)	–0.006	0.011	–0.535	0.594
Type of work (Open field = 0, Greenhouse = 1)	0.064	0.040	1.587	0.117
Use of other protective measures against the sun	0.026	0.035	0.740	0.461
Type of working clothes[a] (Group 1 = 0, Group 2 = 1, Group 3 = 2)	0.006	0.014	0.421	0.675
Use of sunblock (No = 0, Yes = 1)	0.106	0.033	3.203	0.002

[a]Type of working clothes; Group 1: Short-pants and short-sleeved top), Group 2: Short-pants and long-sleeved top or long-pants and short sleeved top, Group 3: Long-pants and long-sleeved top

sunscreen. To our knowledge, however, no studies have been conducted that evaluate the association between UV radiation exposure and urinary TAC. This is because the urinary TAC, unlike the blood TAC, is easily influenced by changes in the environment, diet, or activity. In the present study, we could not investigate other factors that influence the urinary TAC, such as diet and activity. However, the TAC and levels of 8-OHdG in the urine were measured every day for five days, and the average was used. In this way, we expect that the influence of the various confounders was mitigated to some extent.

8-OHdG is a widely used marker for free radical-induced DNA damage, and it is known that various carcinogens, environmental pollutants, and lifestyle factors influence 8-OHdG levels[16–17]. Sunlight or UV radiation has repeatedly been reported to increase 8-OHdG levels in specific animal tissues or cell lines; however, only a few studies have addressed the changes in 8-OHdG levels among people who are exposed to UV in daily-life[21–22]. Recently, in an experiment by Kato *et al.*[23], the use of sunscreen was shown not to affect the urinary 8-OHdG levels after sunlight exposure. Our study showed that the urinary 8-OHdG levels of sunblock users were somewhat higher than those of non-users, but this was not a significant difference. Our results were, therefore, consistent with those of Kato *et al.*, and imply that 8-OHdG in urine is not a suitable marker for UV-blocking effects.

As a pilot study, this investigation had several significant limitations. First, because the number of subjects was very small, the study had the disadvantage of very weak statistical strength. Furthermore, several factors that can affect oxidative stress, particularly diet, were not considered as controls; moreover, selection bias may have occurred because group allocation was not randomized. Sunblock users took additional protective measures (hats, sunglasses, *etc*.) more often than non-users, and they more frequently wore long pants or long-sleeved attire, even during farm work. Hence, we cannot rule out the possibility that the effects of UV-blockage observed in sunblock users were partly due to other factors.

Farmers are known as a vulnerable group in receiving health care service due to various reasons. Especially, Korean farmers tend to accept sun exposure for granted rather than as a hazard factor[24]. However, from the 1980s, Australia and United States have performed 'Slip! Slop! Slap! SunSmart Campaign' focused on multiple ways to avoid the sun, and protect the skin and eyes[25]. The early Slip! Slop! Slap! Campaign promoted 'slipping on a shirt, slopping on sunscreen and slapping on a hat'. When considering the fact that UV exposure from sunlight is increasing gradually due to depletion of the ozone layer and climate change, these campaigns should also be performed actually in East Asian countries including Korea and China like in US and Australia.

In this pilot study, we showed that sunblock is effective in preventing oxidative stress among farmers who work outdoors and that the urinary TAC may be a good marker for determining protective effects against UV radiation. It is necessary to conduct better-controlled, epidemiological studies on a greater number and variety of subjects to corroborate our results. Additionally, our result suggests that it is necessary to conduct active campaigns to protect UV exposure in farmers.

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