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The role of antioxidant and DNA damage in the UVB-induced skin tumors of hairless mice

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Oxidative stress evoked by Ultraviolet (UV) exposure has been suggested to be involved in UV-induced skin carcinogenesis. In this study, the role of oxidative stress in UV-carcinogenesis was evaluated by applying N-Acetylcysteine (NAC) in animal model of hairless-mouse. NAC is known to be a precursor of glutathione, which was converted to glutathione in cytoplasm, acting as an intracellular free radical scavenger. The glutathione levels in hairless mouse skin after one time application of NAC increased significantly. With and without the pre-treatment of NAC, hairless-mice were exposed to UVB three times a week, at total dose 274.4 kJ in 80 times, and the timing of tumor-development, incidence of skin tumor and the histopathology of tumors were observed. 8-hydroxy-2'-deoxyguanosine (8-OHdG), a typical form of oxidative damage in DNA has been also investigated in the course of experiment. The decrease of 8-OHdG formation of UVB-exposed skin compared to controls was observed in the early stage of experiment in the NAC-treated mice. In addition, initial tumor development delayed significantly in NAC-treated group. Finally the number of the tumor developed in the NAC-treated mice was fewer though not significant. These results suggest that antioxidants may have inhibitory effect in the initial step of UVB-induced carcinogenesis of hairless mice.

Key words : glutathione, N-Acetylcysteine, 8-OHdG, UVB, reactive oxygen species

INTRODUCTION

In recent years, various factors have been shown as a reason of carcinogenesis. DNA damage is thought to be one of the most important factors in UV-carcinogenesis [1]. Ultraviolet radiation induces not only direct DNA damages like cyclobutane pyrimidine dimers (CPD) or 6-4 photoproduct but also generates reactive oxygen species (ROS), such as hydroxy radicals (·OH), superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2) by UVB radiation [2,3]. ROS are suggested to act as initiators and promoters in carcinogenesis [4-6]. ROS cause chromosomal damage by direct or indirect action, but the role of this damage in carcinogenesis remains unclear. We have reported reduction of UV-induced skin cancer in mice by topical application of DNA excision repair enzymes, T4 endonuclease V, suggesting that DNA damage play a pivotal role in photo-carcinogenesis [7]. In this study, we investigated the DNA repair activity of a pyrimidine-dimer-specific DNA repair enzyme, T4N5 and a precursor of glutathione, NAC, and the suppressive effect of the agents on UV carcinogenesis of hairless mouse.

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MATERIALS AND METHODS

Chemicals. N-Acetyl-L-cysteine (NAC) was from Wako (Osaka, Japan). Solution of NAC (200mM, or 5% final concentration 238mM)) was prepared in ethanol freshly. T4N5 liposomes, encapsulating purified T4 endonuclease V at the concentration of 1 μg/ml, were kindly provided by Dr. Yarosh, Applied Genetics Inc., Freeport, NY, USA. Control liposomes containing boiled (enzymatically inactive) T4 endonuclease V were also provided.

Animals. BALB/ca-hr hairless female mice, 6-8 weeks old, were obtained from KREA (Japan), housed in plastic cages with wire mesh covers, and fed autoclaved mouse chow and water ad libitum. Room illumination was on an automated cycle of 12-h light, 12-h dark, and room temperature was maintained at 22-25°C.

Immunohistochemistry. Biopsies of normal skin were taken 3days after the 27th, 31st, 43rd, 55th exposure of UVB from representative mice of each group. Immunofluorescence labeling was then performed to detect 8-OHdG as previously [8].

UV carcinogenesis. Four groups including 2 control groups of 15 mice each were irradiated 3 times/week at a dose of 3.43 kJ/m^2 each, unrestrained from above without cage covers, with a bank of 4 Torex FL20SE-30/DMR fluorescent sunlamps (Medical Supply, peak emission at 305nm). Flux intensity was measured with an UVR-305/365D digital radiometer (Opto-Electronic Measuring Instruments). Twenty μL/cm^2 of the NAC solution was applied evenly on the dorsal skin of the mice with a cotton swab 30 minutes before UVB irradiation. The resulting amount of NAC of back skin was about 1.5 μmol/cm^2. T4N5 liposomes lotion was applied immediately after UVB irradiation. Control mice were treated with ethanol or control liposomes. At each exposure, mice were scored for skin tumors in the applied region. A papular lesion > 1mm in diameter was counted as a tumor. At the end of the experiment, all tumors were biopsied and tumor characteristics were pathologically analyzed.

RESULTS

The 8-OHdG index was determined by measuring the positive staining of nuclei in keratinocytes. NAC treatment significantly downregulated the formation until 55th exposures of UVB compared with control or T4N5. The suppressive effect was gradually decreased in the course of experiment, and disappeared at 55th exposures. Interestingly, the expression level of 8-OHdG reached almost plateau from 27th exposures in control or T4N5 group (Fig. 1).

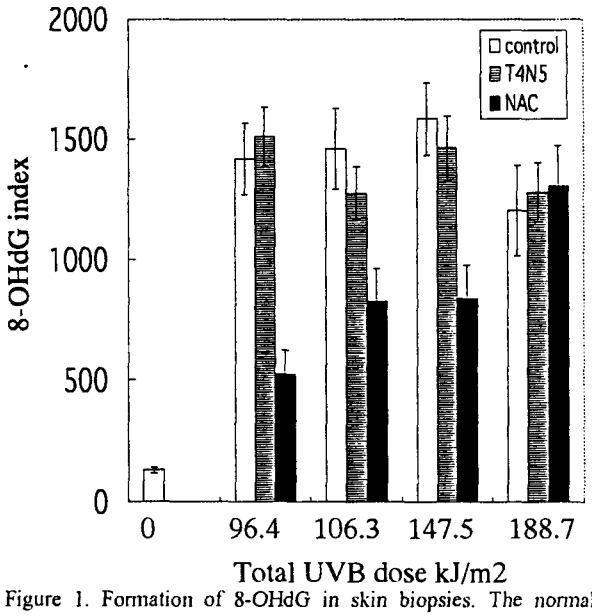

Figure 1. Formation of 8-OHdG in skin biopsies. The normal looking skin of NAC-, T4N5-, or vehicle-treated mice was taken 3days after the 27th, 31st, 43rd, 55th UVB exposures. Immunofluorecent staining was performed for 8-OHdG index using NIH image software.

The expression of the p53 protein was analyzed by

immunohistochemistry. We found the p53 protein overexpression even in the normal looking skin after repeated UVB exposure previously [7]. The p53 protein positive cells were scored in the same timing of 8-OHdG. T4N5 treatment downregulated the p53 protein expression in the epidermis compared with control through the experiment (data not shown). No difference in the p53 positive cell numbers was seen between control and NAC group. As any different reaction was not observed in the mice with ethanol or control liposomes application, the two control groups were taken together into one control group, and 15 mice were selected at random as the control group.

The timing of the first tumor development was compared in each group. The average exposure times to induce the first tumor were 34.9±11.5 in the control group, 52.6±9.8 in the T4N5 group, and 51.9±10.6 in the NAC group. Significant prolongation of tumor development compared with controls was observed in the T4N5- or NAC-treated groups. But finally the incidence of tumors larger than 1 mm in diameter was reduced in the T4N5-treated group compared with NAC-treated or control group (Fig. 2).

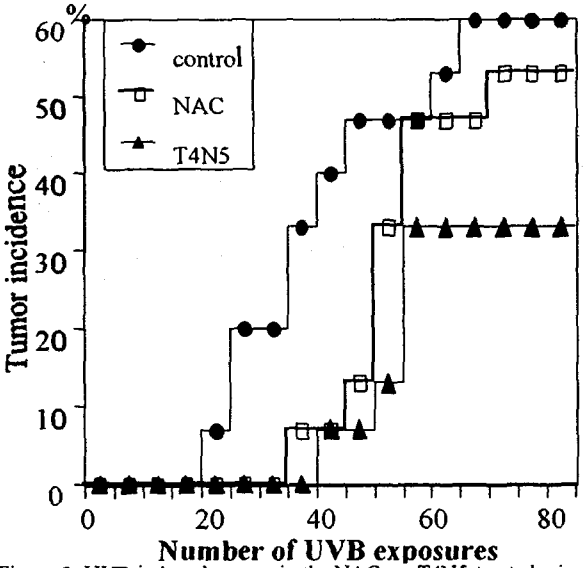

Figure 2. UVB-induced tumors in the NAC- or T4N5-treated mice. BALB/ca-hr hairless mice were exposed to UVB 3 times/week and topically treated 30 minutes before UVB irradiation with NAC solution or immediately afterward with T4N5 liposomes or vehicles. The fraction of mice with at least one tumor larger than 1 mm (tumor incidence) was scored at each irradiation.

All tumors were examined pathologically. The tumors were divided into three classes, which were carcinoma in situ (CIS), differentiated squamous cell carcinoma (SCC), and undifferentiated SCC. Nine CISs, 3 differentiated SCCs, and 2 undifferentiated SCCs were observed in control group. In the NAC-treated mice, 3 CISs, 4 differentiated SCCs, and 3 undifferentiated SCCs were developed. There was no significant difference in tumor pathology between the NAC-treated group and control group. Much less tumors were developed in the T4N5-treated group than the others, and those were 4 CISs and 2 differentiated SCCs.

DISCUSSION

Cellular redox state has been implicated to have an important affect in stress signaling [9]. The direct effect of ROS can alter the conformation and activity of kinase, transcription factor, or DNA. In this study, we generated cysteine-rich condition with the application of NAC in the epidermis of hairless mouse and examined the protective effect of the antioxidant on the UVB-induced stress signaling. The inhibitory effect by NAC on the UVB-induced skin tumors was shown in the initial phase. Recent data suggests that UVB produced ROS, which was associated with DNA damage [10]. Hydrogen peroxide (H_2O_2) and hydroxyl radical are suggested to be involved in UVA- and UVB-induced oxidative DNA damage [11]. NAC treatment suppressed 8-OHdG formation until the 43rd exposures indicating that NAC could partially scavenge the ROS in the early stage of UVB-induced carcinogenesis, and result in the delayed tumor development. Under the presence of H_2O_2, the photodynamic generation of ROS from H_2O_2 with a much

higher efficiency by UVB was observed [12]. The accumulation of ROS and oxidative DNA damage induced by UVB in the late stage might exceed the scavenging effect of NAC and the DNA repair activity. In contrast, T4N5 treatment reduced the UVB-induced tumors in mice significantly compared with the other treatments. The biopsies from the T4N5-treated skin revealed the less frequency of p53 protein expression than the NAC- or vehicle-treated skin (data not shown). These data indicate that the CPD is closely related to UV-induced gene mutation, and the CPD repair contributes to prevent UV-carcinogenesis in mice. The reduction of UV-induced CPD by alpha-tochopherol was observed in mice skin [13]. Preventive effect of p53 mutation was also expected by NAC treatment. But overexpression of p53 protein was detected at the 27th exposures in the NAC-treated skin as well as in control skin, in spite of the decrease of 8-OHdG formation in the NAC-treated skin at the same timing. The CPD formation in the mouse skin may be attributable to the direct DNA damage by UVB, though the role of intracellular ROS should be further clarified.

REFERENCES

1. Ananthaswamy, H. N., W. E. Pierceall (1990) Molecular mechanisms of ultraviolet radiation carcinogenesis. *Photochem. Photobiol.* 52, 1119-1136.

2. Black, H.S. (1987) Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. .*Photochem. Photobiol.* 46, 213-221.

3. Pathak, M. A., K. Stratton (1968) Free radicals in human skin before and after exposure to light. *Arch. Biochem. Biophys.* 123, 468-476.

4. Devary Y., R. A. Gottlieb, T. Smeal, M. Karin (1992) Tha mammalian ultraviolet response is triggered by activation of src tyrosine kinases. *Cell* 71,1081-1091.

5. Troll W., and R. Wiesner (1985) The role of oxygen radicals as a possible mechanism of tumor promotion. *Ann.Rev.Pharmacol.Toxicol.* 25, 509-528.

6. Cerutti P. A. (1985) Prooxidant states and tumor promotion. *Science* 227, 375-381.

7. Bito T., M. Ueda, S. Fujii, M. Ichihashi (1995) Reduction of UV-induced skin cancer in mice by topical application of DNA excision repair enzymes. *Photodermatol. Photoimmunol. Photomed.*11, 9-13.

8. Budiyanto A., N. U. Ahmed, A. Wu, T. Bito, O. Nikaido, T. Osawa, M. Ueda, M. Ichihashi (2000) Protective effect of topically applied olive oil against photocarcinogenesis following UVB exposure of mice. *Carcinogenesis* 21, 2085-2090.

9. Adler V., Yin Z., Tew D. K., Ronai Z. (1999) Role of redox porential and reactive oxygen species in stress signaling. *Oncogene* 18, 6104-6111.

10. Hattori Y., C. Nishigori, T. Tanaka, K. Uchida, O. Nikaido, T. Osawa, H. Hiai, S. Imamura, and S. Toyokuni (1997) 8-Hydroxy-2'-Deoxyguanosine is increased in epidermal cells of hairless mice after chronic ultraviolet B exposure. *J. Invest. Dermatol.* 107, 733-737.

11. Zhang X., S. B. Rosenstein, Y. Wang, M. Lebwohl, and W. Huachen. (1997) Identification of possible reactive oxygen species involved in ultraviolet radiation-induced oxidative DNA damage. *Free. Radic. Biol. Med.* 23, 980-985.

12. Rosen J. E., A. K. Prahald, G. M. Williams (1996) 8-Oxodeoxyguanosine formation in the DNA of cultured cells after exposure to H2O2 alone or with UVB or UVA irradiation. *Photochem Photobiol.* 64, 117-122.

13. Chen W., M. Barthelman, J. Martinez, D. Alberts, H. L Gensler (1997) Inhibition of cyclobutane pyrimidine dimer formation in epidermal p53 gene of UV-irradiated mice by alpha-tocopherol. *Nutr. Cancer* 29, 205-211.