Note

**Supplementing Vitamin B<sub>6</sub> to a Low Vitamin B<sub>6</sub> Diet Exaggerates UVB-Induced Skin Tumorigenesis in DMBA-Treated Hairless Mice**

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**Summary**  
7,12-Dimethylbenz[a]anthracene (DMBA)-treated hairless mice exposed to UVB radiation were used to examine the effect of graded levels of vitamin B<sub>6</sub> [1, 7 or 35 mg pyridoxine (PN) HCl/kg] on skin tumorigenesis for 18 wk. Compared to the 1 mg PN HCl/kg diet, the 35 mg PN HCl/kg diet significantly elevated the incidence and multiplicity of skin tumors, while there was no difference in skin tumorigenesis between the 7 and 35 mg PN HCl/kg diets. Skin levels of oxidative stress markers (lipid peroxides and protein carbonyls) were unaffected by dietary treatment. Compared to the 1 mg PN HCl/kg diet, the 7 and 35 mg PN HCl/kg diets significantly elevated serum pyridoxal 5'-phosphate (PLP) without affecting the skin level of PLP. The results suggest that dietary supplemental vitamin B<sub>6</sub> exaggerates UVB-induced skin tumorigenesis in hairless mice without affecting oxidative stress in the skin.

**Key Words**  
vitamin B<sub>6</sub>, skin tumorigenesis, hairless mice, UVB radiation, oxidative stress

UV radiation results in an increased generation of reactive oxygen species (ROS) that overwhelms the antioxidant defense mechanisms of the skin (1). ROS are associated with the initiation, promotion and progression of skin cancer (2). Vitamin B<sub>6</sub> has been reported to have a strong antioxidant effect like singlet oxygen quencher (3), and to prevent lipid peroxidation (4–6). From these facts, vitamin B<sub>6</sub> might be beneficial for the skin of animals exposed to UV. Recently we have reported the preventive effect of dietary vitamin B<sub>6</sub> in colon and mammary tumorigenesis in rodents treated with carcinogens (7, 8). These effects appeared to be mediated through mechanisms involving suppression in oxidative stress, cell proliferation, inflammation and angiogenesis (9–11). Thus, it is of interest to examine the effect of dietary vitamin B<sub>6</sub> on UV-induced skin carcinogenesis. Our preliminary study has indicated surprising evidence that dietary vitamin B<sub>6</sub> supplementation to a low vitamin B<sub>6</sub> diet enhances UVB-induced skin tumorigenesis in hairless mice. This study was performed to examine the effect of dietary level of vitamin B<sub>6</sub> on UVB-induced skin tumorigenesis, oxidative stress and vitamin B<sub>6</sub> status in hairless mice.

**Materials and methods**

Five-week old female Hos:HR-1 hairless mice (n=45) were purchased from Shimizu Laboratory Supplies (Saitama, Japan). The mice were housed four to a cage at 24±1°C and subjected to a 12:12-h light-dark cycle (lights on, 08:00–20:00 h). Mice were acclimatized for 1 wk before the start of the study, during which time they were fed a commercial stock diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and deionized water. Body weights were measured every week. The animals were maintained according to the “Guide for the Care and Use of Laboratory Animals” established by Hiroshima University. A single application of 190 nmol of DMBA (Sigma Chemical, St. Louis, MO, USA) in 0.2 mL of acetone was painted onto the back of each mouse as the initiator. After 1 wk, the mice were exposed to UVB twice a week for 18 wk. For the first 4 wk, each exposure was 180 mJ/cm<sup>2</sup> UVB, and for the remaining 14 wk, 273 mJ/cm<sup>2</sup> UVB (12). Skin tumor formation was observed weekly, and the tumors of ≥1 mm in diameter were recorded if they persisted for 2 wk or more.

The experimental feeding period began at the same time as UVB exposure. The mice were randomly divided into three groups of 15 animals. Composition of the basal diet was described elsewhere (7). Vitamin B<sub>6</sub> (PN HCl, Nacalai Tesque, Inc., Kyoto, Japan) was supplemented to the basal diet at the levels of 1, 7 and 35 mg/kg diet, constituting three dietary groups. The level of PN HCl recommended in the AIN-93 diets is 7 mg/kg diet (13), and a 1 mg/kg diet has been reported to be the minimum level required for preventing growth depression caused by vitamin B<sub>6</sub> deficiency (14). The experimental feeding period was 18 wk. Twenty-four hours after the last UVB exposure, the mice were killed by decapitation under anesthesia with diethylether.

At the termination of the experiment, the skin tissues were removed and immediately fixed in 10% neutral-
buffered formalin, and embedded in paraffin. Biopsied tumors were sectioned to 4 μm thickness and used for pathological examination. Normal skin tissues around tumors were used for the determination of lipid peroxide and protein carbonyls (protein oxidation). Skin level of lipid peroxide (thiobarbituric acid reactive substances: TBARS) was determined by the method described by Ohkawa et al. (15). Tetramethoxypropane was used as an external standard. The protein content of skin homogenates for the assay of lipid peroxide was determined using the DC protein assay kit from Bio-Red (Hercules, CA, USA), with bovine serum albumin as a standard. Skin protein peroxidation (protein carbonyls) was determined by a modified DNPH-based method described by Patsoukis et al. (16). In this assay of protein carbonyls, extracted protein levels were estimated spectrophotometrically with the absorbance at 280 and 260 nm (16). Serum and skin concentrations of pyridoxal 5′-phosphate (PLP) were determined by the HPLC method described elsewhere (17). Values are presented as means±SE. Statistical difference among tumor incidence was examined according to the Kaplan-Meier method, and other data were analyzed by one-way ANOVA and Scheffe’s multiple-range test (Excel Statistics 2006 for Windows, Social Survey Research Information Co., Ltd., Tokyo, Japan). Statistical significance was estimated at p<0.05.

Results
The mice gained weight at similar rates, and there was no difference in the final body weight (g) among the 1, 7 and 35 mg PN HCl/kg diet groups (36.2±1.0, 35.5±0.9 and 34.4±0.7, respectively, p>0.05). Food intake for 4 d at weeks 3, 7 and 18 was unaffected by dietary level of vitamin B₆ (data not shown). In the 1 mg PN HCl/kg diet group, no depression in the growth or food intake was observed during the experimental period, implying no symptoms of vitamin B₆ deficiency in the 1 mg PN HCl/kg diet group.

The tumors in each group of mice were histologically identified as papillomas. After dissection, no tumors were observed in other tissues or organs. At weeks 17 and 18, tumor incidence (percentage of mice with tumors) in the groups fed 35 and 7 mg PN HCl/kg diet was 73 and 67%, respectively, compared to 33% in the group fed 1 mg PN HCl/kg diet (Fig. 1A). Analysis by the Kaplan-Meier method estimated a significant difference in the increase of tumor incidence over the time course of UVB irradiation between the 35 and 1 mg PN HCl/kg diet groups (p<0.05, Fig. 1A). At weeks 18, tumor multiplicity in the 35 mg PN HCl/kg diet group was significantly higher compared with the 1 mg PN HCl/kg diet group (1.41±0.27 and 0.47±0.19, respectively, p<0.05, Fig. 1B), while that in the 7 mg PN HCl/kg diet group (1.00±0.24) was not significantly different from the 1 mg PN HCl/kg diet group (p>0.05). At weeks 18, the tumor sizes were unaffected by dietary manipulation (data not shown). Skin levels of TBARS and protein carbonyls did not differ significantly among the three groups (Table 1). Serum PLP was higher in the 7 mg PN HCl/kg diet group compared to the 1 mg PN HCl/kg diet, while the skin concentration of PLP was unaffected by dietary treatment.

Discussion
This study indicated that dietary supplementation of
vitamin B₆ to a low vitamin B₆ diet potentiates UVB-induced skin tumorigenesis. Our study indicates that compared with the low vitamin B₆ diet (1 mg PN HCl/kg diet), UVB-induced skin tumorigenesis is enhanced by the 35 mg PN HCl/kg diet, which is far lower than any acute toxic level (>500 mg PN HCl/kg diet) (18). Our finding implies that supplementation of vitamin B₆ to a low vitamin B₆ diet is able to cause adverse effect for skin health under the condition of UV exposure. Our data indicated that the dietary level of vitamin B₆ (range from 1 mg to 35 mg PN HCl/kg diet) caused no influence on skin PLP level, while serum PLP was significantly lower in the 1 mg PN HCl/kg diet than in the 7 mg PN HCl/kg diet. Consistent with this result, compared with the 7 mg PN HCl/kg diet, the 1 mg PN HCl/kg diet caused no influence on the levels of PLP in extra hepatic tissues of ICR mice such as muscle, brain and lung, but significantly reduced serum and hepatic PLP (Kato et al., unpublished data). Thus, the possibility that the stimulating effect of supplemental vitamin B₆ on UVB-induced skin tumorigenesis is mediated by skin PLP level appears to be negated. Possibly, the stimulating effect of vitamin B₆ might be at least in part ascribed to factors relating to serum and hepatic PLP.

Recently, vitamin B₆ has been found to have a strong antioxidant effect like a singlet oxygen quencher (2). Therefore, we expected that higher intake of vitamin B₆ to suppress skin tumorigenesis through lowering oxidative stress. However, against our expectation, our study indicated higher skin tumorigenesis with higher intake of vitamin B₆, though skin oxidative stress markers (TBARS and protein carbonyls) were unaffected by dietary treatment. Recently our group has studied the influence of dietary vitamin B₆ on DMBA-treated, 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced skin tumorigenesis in ICR mice (Lu et al., unpublished data). There was no significant difference in the tumor development among the groups fed 1, 7 and 35 mg PN HCl/kg diets in TPA-induced skin tumorigenesis. Thus, a higher skin tumorigenesis with a higher vitamin B₆ intake appears to be observed in the UVB-induced, but not in the TPA-induced skin tumorigenesis. Taken together, these results suggest that an interaction between vitamin B₆ and UVB radiation might be responsible for the higher carcinogenic process in skin.

It has been reported that excessive exposure to vitamin B₆ can develop UVA-induced photosensitivity in humans (19) and in cultured fibroblasts (20–22). The cytotoxic effects of UV-irradiated B₆ vitamers include inhibition of cell proliferation, and elevation of protein photocross-linking and peptide photoproteinization (22). Although ROS formation appears not to be essential for the skin phototoxicity of B₆ vitamers (22), the exact mechanism of the phototoxicity of vitamin B₆ is still unclear. It is of importance to test if the higher development of UVB-induced skin tumors with higher intake of vitamin B₆ observed in this study is a result of the phototoxicity of vitamin B₆. Further study is in progress to define the underlying mechanisms of the higher skin tumorigenesis by vitamin B₆.

Vitamin B₆ was originally discovered as an anti-dermatitis factor, and has been believed to be essential for skin development and maintenance (23, 24). However, surprisingly, this study indicated that a higher intake of vitamin B₆ is associated with higher UV-induced skin tumorigenesis. Our finding raises a fundamental question whether a higher intake of vitamin B₆ is favorable for skin health under UV exposure.

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