Promotion of Skin Carcinogenesis by Dimethylarsinic Acid in *Keratin (K6)/ODC* Transgenic Mice

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Dimethylarsinic acid (DMA) is a major metabolite of inorganic arsenicals in mammals, and arsenic exposure is associated with tumor development in a wide variety of human tissues, particularly the skin. Transgenic mice with ornithine decarboxylase (ODC) targeted to hair follicle keratinocytes are much more sensitive than littermate controls to carcinogens. In this study we investigated the promoting effect of DMA on skin carcinogenesis in such *K6/ODC* transgenic mice. The back skin of female C57BL/6J *K6/ODC* transgenic mice, 10 to 14 weeks old, was initiated with topical application of 7,12-dimethylbenz[a]anthracene (DMBA) at a dose of 50 µg or acetone alone on day 1 of the experiment, followed by treatment with 3.6 mg of DMA, 5 µg of 12-O-tetradecanoylphorbol-13-acetate (TPA) or neutral vehicle (control) twice a week for 18 weeks. Mice were killed 1 week after the end of the treatment. Induction of skin tumors was significantly accelerated in the DMA-treated group, as well as in the TPA-treated group, indicating that DMA has a promoting effect on skin tumorigenesis in *K6/ODC* transgenic mice.

Key words: *K6/ODC* transgenic mice — ODC — DMA — Skin carcinogenesis

Arsenic is a ubiquitous trace element, widely distributed in the environment, and is epidemiologically related to certain human cancers.\(^1\)\(^{-2}\) Large numbers of residents who consumed high-arsenic artesian well water developed cancers of the skin, lung, urinary bladder and kidney in Taiwan.\(^3\)\(^{-6}\) At present many people are exposed to geochemical inorganic arsenic in well water in China, Bangladesh and the Bengal district of India, and the resultant occurrence of skin cancer is likely to be a problem in the future. Dimethylarsinic acid (DMA) is the major methylated metabolite of inorganic arsenic, and a potent clastogenic agent, causing gene amplification.\(^7\)\(^{-8}\) Yamamoto *et al.*\(^9\) recently found, using an *in vivo* multi-organ carcinogenesis model, that DMA promoted carcinogenesis in the urinary bladder, kidney, liver and thyroid gland of rats initiated by sequential treatment with 5 carcinogens. Wanibuchi *et al.*\(^10\)\(^{-11}\) have shown that DMA exerts dose-dependent promoting effects on urinary bladder and liver carcinogenesis in rats, possibly via a mechanism involving stimulation of cell proliferation, in which DNA damage caused by oxygen radicals may be involved. Wei *et al.*\(^12\) found that DMA induces carcinomas of the urinary bladder in F344 rats, when administered in the drinking water for a 2-year period. Moreover, DMA enhanced spontaneous tumor induction in *p53* knockout mice in a one and a half year study, while not showing organ-specific carcinogenicity (unpublished data). Whether it is a complete carcinogen in the skin remains uncertain. Mouse skin tumorigenesis can be generally divided into two distinct stages of tumor initiation and promotion, resulting from topical application of an initiator such as 7,12-dimethylbenz[a]anthracene (DMBA), followed by repeated treatment with a promoter.\(^13\)\(^{-14}\) 12-O-Tetradecanoylphorbol-13-acetate (TPA) is the most widely studied skin tumor promoter.

Ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis, increases concomitantly with epithelial cell proliferation of skin when tumor promoters are administered to rodents,\(^15\) and this apparently increases tissue susceptibility to tumor development in the mouse.\(^16\) The *K6/ODC* transgenic mouse model has been very useful in establishing the importance of polyamines in influencing susceptibility to carcinogenesis.\(^17\) In this mouse line, a bovine K6 promoter/regulatory region drives expression of a truncated ODC protein in outer root sheath keratinocytes of the hair follicle. The transgene is maintained in the hemizygous state by breeding of hemizygous males with C57BL/6J females, resulting in production in approximately equal numbers of transgenic pups and normal littermates.\(^18\)\(^{-19}\) In the present study, we have examined the tumor-promoting activity of DMA in *K6/ODC* transgenic mice using a two-stage carcinogenesis protocol. TPA was also examined as a positive control.

Twenty-four female C57BL/6J *K6/ODC* transgenic mice obtained at 10–14 weeks of age (The Jackson Laboratory, Bar Harbor, ME) were housed in an air-conditioned...
room at a temperature of 23±1°C and relative humidity of 60±5%, with a 12 h light-12 h dark cycle. The animals had free access to feed (CE2; Clea Japan, Tokyo) and tap water. DMBA and DMA were obtained from Wako Pure Chemical Industries (Osaka).

Twenty-four mice were divided into 4 groups (groups 1 and 2, 7 mice each; group 3, 8 mice; group 4, 2 mice) for the initiation/promotion protocol. A single dose of 50 µg of DMBA dissolved in 200 µl of acetone was topically applied to the dorsal skin as an initiator in groups 1, 2 and 3; acetone alone was used as a vehicle control in group 4. Starting 1 week after initiation, a dose of 5 µg of TPA dissolved in 200 µl of acetone was applied to the dorsal skin twice weekly in group 1. A dose of 3.6 mg of DMA dissolved in neutral cream was similarly applied to the dorsal skin after initiation in groups 2 and 4, while neutral cream alone was used as a control in group 3. These treatments were continued for 18 weeks. The body weights of mice were measured once every week. The incidences and numbers of tumors on treated areas of the skin were assessed every week and those at least 1 mm in diameter were counted. All mice were killed 1 week after the end of promotion treatment by exsanguination under ether anesthesia to give a total observation period of 20 weeks. After counting of grossly visible lesions, the skin was fixed in 10% formalin for hematoxylin and eosin staining and histological examination.

Statistical analyses were performed using Student’s t test (Stat View SE+Graphics, Abacus Concepts, Inc., Berkeley, CA). P values of 0.05 or less were considered to be significant.

All mice survived until week 20 and maintained a relatively healthy appearance throughout the experiment. Average body weight curves (shown in Fig. 1) did not show any statistically significant variation among the four groups. The macroscopic incidences of skin tumors are summarized in Fig. 2. In group 1, skin tumors began to appear at 7 weeks, and all mice had developed skin tumors after 11 weeks. In group 2, the respective figures were 8 and 14 weeks, and in group 3, 11 and 16 weeks. In group 4, treated with acetone followed by DMA, no tumors were observed. Data for average numbers of skin tumors per mouse are summarized in Fig. 3. The average numbers of tumors in groups 1 and 2 were significantly increased to a similar extent, compared to those in group 3, from 10 to 20 weeks after initiation. At 20 weeks after initiation, the average numbers of tumors per mouse were 20.7±8.4, 19.4±10.2, and 9.7±3.5 in groups 1, 2, and 3, respectively. Macroscopically, well-demarcated papules or nodules, about 1 to 30 mm in diameter, were sporadically observed.
in groups 1, 2 and 3. Microscopically, most tumors were squamous papillomas, although some in groups 1 and 2 were squamous cell carcinomas showing a disorderly arrangement, and nuclei often appeared atypical and pleomorphic. The skin of the non-treated transgenic mice showed degeneration of hair follicles and formation of follicular cysts in the dermis. Skin tumors appeared to originate from the cells lining the follicular cysts.

In the K6/ODC transgenic model, high-level expression of an ODC transgene driven by a K6 promoter/regulatory sequence is a condition that renders the animals very sensitive to skin tumor induction by several carcinogens.\(^1\) In the present two-stage skin carcinogenesis study, TPA and DMA accelerated the development of tumors, with an increase in yield, compared to that in vehicle-treated mice. Without prior carcinogen exposure, DMA exerted no obvious effect. These results indicate that DMA is a tumor promoter, like TPA, in K6/ODC transgenic mice. Recently, Katsumata et al.\(^2\) have reported a promoting effect of DMA on UVB-induced mouse skin tumorigenesis, in line with our present results. The data add weight to the implication of a human risk of skin tumors after exposure to arsenicals.

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