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SHORT COMMUNICATION

Mouse skin papilloma formation by chronic dermal application of 7,12-dimethylbenz[*a*]anthracene is not reduced by diet restriction

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Diet restriction has repeatedly been shown to reduce the incidence of spontaneous and chemically induced tumors in rodents. However, no conclusive data are available to show whether carcinogenesis by chronic exposure to a genotoxic agent can also be retarded. In this study, diet restriction to 70% was investigated for a protective effect on the formation of skin papilloma in male NMRI mice treated twice weekly with 20 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA). Rather surprisingly, no protection was seen. Both time of onset of papilloma formation (13 weeks in both groups) and time of 50% cumulative incidence (t_{50}; 17.5 and 18 weeks) were similar in the unrestricted and the restricted group. In contrast, a clearly protective effect was found in mice initiated with 100 nmol DMBA and promoted twice weekly with 2.5 nmol 12-*O*-tetradecanoylphorbol-13-acetate: the onset of papilloma formation increased from 7 to 11.5 weeks, the t_{50} was shifted from 8.5 to 19 weeks. Diet restriction, therefore, was not protective under conditions of chronic exposure to a genotoxic carcinogen. It cannot be considered a universal measure of cancer prevention.

A large number of experimental studies have demonstrated that diet restriction markedly reduces both spontaneous and chemically induced tumor formation in mice and rats (1–17). A number of reviews are also available (18–21). Reports on experiments where restriction had no protective effect are rare (10). It therefore appears as if reduction of food intake could be regarded as a universal cancer-preventive measure.

In the majority of the investigations on chemically induced tumor induction, the carcinogen was given as a single dose (10,12–14) or for up to 4 weeks (5,7,10,15). In few studies, the treatment with the carcinogen lasted longer but was stopped either before the appearance of the first tumor (2–4) or when the first tumor appeared in the unrestricted group (1). It therefore remains to be shown whether the process of carcinogenesis can be retarded by diet restriction under conditions of chronic administration of a genotoxic agent. To answer this question, the skin tumor model with NMRI mice and chronic administration of 7,12-dimethylbenz[*a*]anthracene (DMBA*) was used (22–24). Restriction was by feed reduction to 70%, a level chosen on the basis of the findings that underfeeding at a level of 60% was well tolerated and indistinguishable from caloric (carbohydrate) restriction to 60% with respect to body weight and tumor incidence (16).

Male NMRI mice [Crl:NMRI BR] were obtained from Charles River Savo, Kisslegg, Germany, at 6 weeks of age. The mice were housed individually at 21 ± 1°C with a 12 h light/dark cycle in Macrolon cages type II. Feed (Nafag 890 from Nafag AG, Gossau, Switzerland) and water were given *ad libitum* to all mice from 6 to 8 weeks of age. The backs of the mice were shaved at week 7 (a swatch 4 cm long, 3 cm wide; Wella Minicut). One week later, restriction and treatment was started. The restricted mice were given weighed feed portions amounting to 70% of the feed eaten by the unrestricted group. Every evening at 6.30 p.m., i.e. 30 min before the beginning of the dark phase, a rotating wheel delivered the weighed portions into the feed compartment of the cages. The size of the portions was adjusted weekly on the basis of the weighed feed intake by the unrestricted group.

Treatment A (chronic administration of DMBA): starting at week 0, 60 mice (30 *ad libitum*, 30 restricted) received twice weekly a dermal application of 20 nmol DMBA (Sigma) in 0.1 ml acetone on the shaved part of the back until 4 days before they were killed. Application solution was prepared biweekly and was stored at −20°C. One mouse in each group died spontaneously during the experiment. Treatment B (initiation with DMBA, promotion with 12-*O*-tetradecanoylphorbol-13-acetate [TPA]; positive control for a protective effect of diet restriction): twenty-eight mice (14 *ad libitum*, 14 restricted) were treated once with 100 nmol DMBA in 0.1 ml acetone (= week 0). Treatment with 2.5 nmol TPA (LC Services Corporation, Woburn, MA), twice weekly in 0.1 ml acetone, was started 1 week later. A TPA stock solution (8 ×) was prepared once, divided into 5 ml aliquots and stored at −20°C. Two mice of the restricted group died spontaneously during the experiment. Controls: two groups of mice (9 *ad libitum*, 9 restricted) were treated twice weekly with 0.1 ml acetone.

All mice were visually inspected and palpated for papillomas twice weekly and were weighed biweekly. They were killed 2 weeks after showing the first, persistent papilloma with diameter >1 mm.

Body weights are given in Figure 1. Diet restriction resulted in a significant reduction in body weight under both treatments. In the initiation–promotion model (Figure 1; treatment B), the initial decrease appeared to be faster, probably because of some high-dose effect of the initiating 100 nmol DMBA. The initiation might also be the reason why the weight gain in the unrestricted group of treatment B was slower than in treatment A. The weight curves for the control groups treated with acetone alone were not different from those seen with treatment A (data not shown).

The cumulative skin-papilloma incidence is shown in Figure 2. Unexpectedly, diet restriction had no protective effect when the mice were treated chronically with DMBA (Figure 2A). Both time of onset of papilloma formation (13 weeks) and time of 50% cumulative incidence (t_{50}; 17.5 and 18 weeks) were similar in both groups. A papilloma incidence of 100% was reached after 25 and 28 weeks in unrestricted and restricted animals respectively.

In contrast, and as expected from the literature (16), diet restriction had a clearly protective effect in the initiation–

*Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; B[*a*]P, benzo[*a*]pyrene.

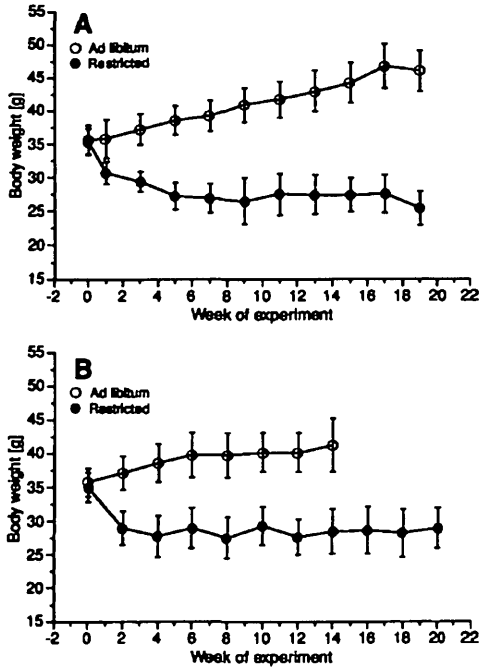

Fig. 1. Body weights of male NMRI mice fed *ad libitum* (open circles) or restricted to 70% feed intake (full circles). Treatment A (group size at start: 30): 20 nmol DMBA twice weekly, starting at week 0. Treatment B (group size at start: 14): initiation with 100 nmol DMBA at week 0, followed by promotion with 2.5 nmol TPA twice weekly, starting at week 1. Data are given as mean weights ± 1 SD.

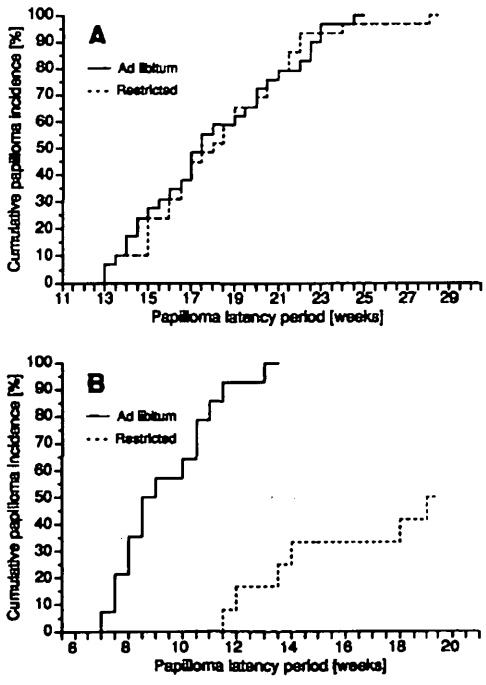

Fig. 2. Cumulative incidence of skin papillomas in male NMRI mice fed *ad libitum* (solid line) or restricted to 70% feed intake (dashed line). Treatment A: 20 nmol DMBA twice weekly, starting at week 0. Treatment B: initiation with 100 nmol DMBA at week 0, followed by promotion with 2.5 nmol TPA twice weekly, starting at week 1.

promotion model (Figure 2B). The onset of papilloma formation increased from 7 to 11.5 weeks, the t_{50} was shifted from 8.5 to 19 weeks. The papilloma incidence in the unrestricted group was 100% after 13 weeks and it would have taken much longer to reach 100% in the restricted group. Histological examination of the papillomas did not reveal any observable differences between the four groups. In the acetone control groups, no tumor was recorded within 28 weeks of observation. For controls treated only with DMBA (1×100 nmol) or TPA (10 nmol twice weekly), the papilloma incidence within 24 weeks had been reported to be 0 or 4% (22).

Our negative data are in contrast to Tannenbaum's early findings mentioned above (1–4). The discrepancy could be due to the use of different carcinogens and different dose levels. The 5 μg DMBA dose used here and the 60 μg benzo[*a*]pyrene (B[*a*]P) dose used by Tannenbaum (1,3) can be considered equicarcinogenic (25,26) but the relative importance of genotoxic versus promoting activity associated with the repeated administration of the two carcinogens could be different. For DMBA, carcinogenicity might be dominated by genotoxicity while the 10-fold higher chemical dose of B[*a*]P could include a more pronounced 'promotional' activity. Such an explanation would also be in line with recent work by Birt and co-workers who showed that diet restriction had a beneficial effect only when applied during tumor promotion but not during initiation (16).

It could be argued that a dose of 20 nmol DMBA twice weekly was too high to allow restriction to come into play. The possibility that high doses of carcinogen could override the effects of diet restriction was mentioned by Tannenbaum and Silverstone in their review article (18). However, the dose used here did not result in a maximum rate of the process of carcinogenesis. While we observed a median latency time of 17.5 weeks, dose levels of 25, 50 and 100 nmol resulted in t_{50} values of 16, 12.5 and 11 weeks respectively (24). Furthermore, the initiation–promotion protocol used in treatment B resulted in an even faster appearance of the papillomas in the unrestricted group and still allowed diet restriction to be protective. The question of whether diet restriction can retard the process of papilloma formation therefore strongly depends on the mechanism of action. It appears as if clonal expansion of initiated cells during tumor promotion could be reduced by diet restriction but that the rate of accumulation of critical mutations from chronic exposure to a genotoxic agent is not affected.

Our results suggest that diet restriction is not protective under all circumstances of chronic exposure to a carcinogen. Whether or not there is an effect depends upon the specific mechanism of carcinogenesis. For a situation of chronic high-dose exposure of mouse skin to a genotoxic agent the formation of papilloma could not be reduced. Although the data do not give any information on the process of tumor progression to carcinoma, they strongly indicate that diet restriction should not be considered a universal cancer-preventive measure.

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