Inhibition of 7,12-dimethylbenz[a]anthracene-induced lung tumorigenesis in A/J mice by food restriction is reversed by adrenalectomy

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Prior work has demonstrated that food restriction of mice markedly suppresses 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion of skin papillomas and adrenalectomy prior to initiating food restriction completely reverses the tumor inhibitory effect of underfeeding. In the present experiment the effect of food restriction, with or without prior adrenalectomy, on 7,12-dimethylbenz[a]anthracene (DMBA)-induced lung tumor development in A/J mice was explored. Food restriction (27%), beginning 3 weeks after a single oral dose of 0.5 mg DMBA and continued for the duration of the experiment (14 weeks), significantly inhibited lung adenoma development, whereas adrenalectomy 2 weeks before initiating food restriction abolished the tumor inhibitory effect of underfeeding and also enhanced tumor development in the ad libitum fed mice. Plasma corticosterone levels were significantly elevated in food-restricted A/J mice, whereas plasma dehydroepiandrosterone (DHEA) levels showed no apparent change. These studies suggest that adrenal gland secretory products may play a general role in the tumor preventive effect of food restriction in laboratory mice.

Materials and methods

Induction of tumors and adrenalectomy

Male A/J-Jax mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 7 weeks of age. Mice were initially housed in the Fels Animal Facility at five animals per cage in 11.5 x 7 x 5 inch plastic shoebox cages on hardwood bedding with ad libitum access to Purina 5015 chow. After 1 week acclimatization to the facility mice were treated per os with 0.5 mg DMBA (ChemSyn Laboratories, Lenexa, KS) in 0.2 ml sesame oil. All mice were treated with DMBA during the hours of 1 and 3 p.m.

One week after DMBA treatment the mice were either adrenalectomized or sham operated. The mice were anesthetized with Mesofane and a midline incision was made on the back of each mouse. The skin was loosened from the abdominal wall, an incision was made in the abdominal wall on each side and the adrenal glands were removed. The abdominal wall was sutured with 3.0 mm gut suture material and the outer skin was closed with 9 mm wound clips. Sham-operated mice were treated identically except that the adrenal glands were not removed. The mice were given 0.5 ml 0.9% NaCl i.p. to minimize the effect of dehydration due to surgery. Mice were kept warm (each cage was placed on a heating pad) following surgery to minimize hypothermia. Each mouse was kept in a cage on a heating pad until it was moving freely after surgery. Adrenalectomized mice were given ad libitum access to 1% NaCl immediately after surgery and throughout the remainder of the experiment. Following adrenalectomy or sham operation all mice were singly housed in 9.4 x 5.4 x 5.1 inch plastic shoebox cages for the duration of the experiment.

Food restriction

Groups of mice were started on a food restriction regimen 2 weeks after adrenalectomy or sham operation. Mice were divided into four groups: (i) sham-operated, ad libitum fed, 30 mice; (ii) sham-operated, food-restricted, 33 mice; (iii) adrenalectomized, ad libitum fed, 32 mice; (iv) adrenalectomized, food-restricted, 43 mice. Food consumption was determined weekly, with correction made for food lost in the bedding, as described previously (15). The mice were weighed approximately biweekly around the Sartorian balance (model 1409-M8-1) equipped with an animal weighing program. Food-restricted mice received daily allotments at -11:30 a.m. of Purina chow that were 27% less than the mean quantity of food consumed by the ad libitum fed groups.

Animals and methods

Fourteen weeks after DMBA treatment mice were killed with an overdose of CO2. The lungs were excised, rinsed in 0.9% NaCl and fixed in Formalin. Fourteen weeks after DMBA treatment mice were killed with an overdose of CO2. The lungs were excised, rinsed in 0.9% NaCl and fixed in Formalin. Five ml glacial acetic acid, 100 ml 70% alcohol), which brings out clearly the smallest tumor nodules in the lung (16). Tumors were counted with the aid of Bausch and Lomb 3.5X and 7X hand magnifiers.

We performed a square root transformation on the number of tumors in each group in order to more closely approximate the normality assumption required by classical ANOVA. The four groups are the treatment combinations of two factors each at two levels: sham-operated versus adrenalectomized and ad libitum versus food-restricted. The data were then analyzed using the program PROC GLM of SAS statistical software (17).

Abbreviations: TPA, 12-O-tetradecanoylphorbol-13-acetate; DHEA, dehydroepiandrosterone; DMBA, 7,12-dimethylbenz[a]anthracene.
Table I. Effect of food restriction and adrenalectomy on lung tumor development

<table>
<thead>
<tr>
<th></th>
<th>Number of mice</th>
<th>Lung tumors (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated, ad libitum fed</td>
<td>30</td>
<td>2.03 ± 0.29</td>
</tr>
<tr>
<td>Sham-operated, food-restricted</td>
<td>29</td>
<td>0.52 ± 0.10</td>
</tr>
<tr>
<td>Adrenalectomized, ad libitum fed</td>
<td>28</td>
<td>4.46 ± 0.83</td>
</tr>
<tr>
<td>Adrenalectomized, food-restricted</td>
<td>26</td>
<td>4.65 ± 0.97</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of the total number of lung tumors per mouse for each group. Virtually all of the tumors examined histologically (96%) were type II alveolar adenomas.

*Significantly greater than sham-operated, food-restricted, P < 0.008.

**Significantly greater than sham-operated, ad libitum fed, P < 0.007.

***Significantly greater than sham-operated, food-restricted, P < 0.001.

Plasma corticosterone and DHEA levels

In order to assess the effect of food restriction on plasma corticosterone and DHEA levels, 6-week-old A/J-lax mice were obtained from the Jackson Laboratory. After 2 weeks of acclimatization to the Fels Animal Facility mice were individually housed and either fed ad libitum or food restricted to ~32% less food than the ad libitum fed group.

After 36 days of ad libitum feeding or food restriction mice were bled between the hours of 3 and 5 p.m., a time when plasma corticosterone levels are significantly elevated from the diurnal nadir (18). To minimize stress to the mice on the day of plasma collection no personnel entered the animal room prior to bleeding. Mice were removed from their cages, lightly anesthetized with Metofane and bled from the orbital sinus using heparinized Natelson blood collecting tubes. All mice were bled within 2 min of removal from the cage. Blood (~250 µl from each mouse) was pooled from four mice for each sample. Four samples were analyzed in each group (total of 16 mice). Blood was kept on ice until the bleeding was finished. The samples were then centrifuged at 3000 g for 15 min. The plasma was transferred to 1 ml plastic tubes, frozen in liquid nitrogen and stored at -80°C.

Frozen samples were sent to Endocrine Sciences (Tarzana, CA) for determination of corticosterone levels by radioimmunoassay and to Dr. Gary Gordon (Johns Hopkins University Medical School) for determination of DHEA and DHEA sulfate levels by radioimmunoassay (19).

Histological examination of tumors

For histological examination of tumors the lungs were trimmed, embedded in paraffin, sectioned to a thickness of 5 µm and stained with hematoxylin and eosin. Individual tumors were characterized as alveolar or bronchiolar adenomas by morphological criteria (20). Tumor examination was performed by Dr. Thomas Van Winkle (University of Pennsylvania School of Veterinary Medicine). A total of 28 lungs were examined (eight adrenalectomized, ad libitum fed; seven adrenalectomized, food-restricted; seven sham-operated, food-restricted; six sham-operated, ad libitum fed).

Results

As shown in Table I, food restriction of sham-operated animals significantly inhibited lung tumor development (P < 0.008). Adrenalectomy of mice 1 week after DMBA treatment and 2 weeks prior to initiating food restriction completely eliminated suppression of lung tumor development produced by food restriction. Adrenalectomy also enhanced the number of lung tumors observed in the ad libitum fed animals (P < 0.007). Droms et al. (21) also found that adrenalectomy of ad libitum fed A/J mice significantly enhanced the number of lung adenomas produced by a single injection of urethane.

Histological analysis of tumors revealed that virtually all the tumors were type II alveolar adenomas. Of a total of 49 tumors examined 47 were alveolar adenomas and two were bronchiolar adenomas (one in the adrenalectomized, food-restricted group and one in the sham-operated, ad libitum fed group).

At the termination of the experiment, 14 weeks after DMBA treatment, the numbers of mice alive in each group were: (i) sham-operated, ad libitum fed, 100% (30/30); (ii) sham-operated, food-restricted, 88% (29/33); (iii) adrenalectomized, ad libitum fed, 88% (28/32); (iv) adrenalectomized, food-restricted, 60% (26/43). The increased mortality of the adrenalectomized, food-restricted A/J mice is similar to that experienced by CD-1 mice (8) and reflects the greater fragility of these animals to the stress of food deprivation.

Although the ad libitum fed, sham-operated animals consumed the same amount of food as the ad libitum fed, adrenalectomized mice, the adrenalectomized mice gained less weight. In contrast, the adrenalectomized, food-restricted mice gained more weight than their non-adrenalectomized, food-restricted counterparts (Figure 1). The reason for these alterations in the efficiency of food utilization in the various treatment groups is not clear.

As shown in Table II, plasma corticosterone levels were significantly greater than ad libitum fed, P < 0.001, Student's t-test.
significantly elevated in A/J mice food restricted for 36 days; DHEA plasma levels, in contrast, showed no apparent change.

Discussion

These experiments demonstrate that adrenalectomy prior to initiating food restriction reverses the tumor inhibitory effect of underfeeding in a mouse lung tumorigenesis model; together with a similar finding in a mouse skin tumor model, these results suggest that secretory products of the adrenal gland may play a major role in mediating the tumor inhibitory effect of food restriction.

Plasma corticosterone levels (blood drawn between 3 and 5 p.m.) were 2.7-fold higher in the A/J food-restricted mice compared with ad libitum fed animals. We also observed a 1.7-fold elevation in plasma corticosterone levels (taken between 9 and 11 a.m.) in CD-1 mice which received 27% less food than ad libitum fed controls for 11 weeks (8), as well as a 3.3-fold elevation in plasma corticosterone levels (taken between 9 and 11 a.m.) in CD-1 mice receiving 43% less food than ad libitum fed controls for 8 weeks (unpublished observation). Klebanov et al. (18) found that 40% food restriction of BALB/c mice for 4-6 weeks produced a 3-fold rise in the diurnal elevation of plasma corticosterone levels, as well as an attenuated inflammatory response in hindfoot pads injected with carrageenan.

A/J mice that were food restricted and adrenalectomized experienced a greater mortality throughout the lung tumorigenesis experiment than mice which were food restricted and sham operated. We observed a similar effect of adrenalectomy on survivorship of food-restricted CD-1 mice (8). A critical role of glucocorticoid hormones is to maintain glucose homeostasis during periods of food deprivation (22) and this may account for the increased fragility of food-restricted mice lacking adrenal glands.

In previous experiments we found that the hyperplastic response of mouse epidermis to topical treatment with the tumor promoter TPA is abolished by 1 week of food restriction prior to TPA treatment (8,23). This anti-hyperplastic effect of food restriction is very likely critical to the anti-tumor promoting action of underfeeding in the mouse skin, since it is likely that the hyperproliferative and tumor promoting actions of TPA are causally linked (24).

We have quantitated the degree of epidermal hyperplasia produced by TPA treatment by either measuring the specific incorporation of [3H]thymidine into epidermal DNA 20 h after TPA application or by determining the epidermal DNA content of a 2×2 cm² section of mouse skin 48 h after TPA treatment. Using both of these measurements of epidermal hyperplasia 1 week of food restriction prior to a single topical TPA application completely abolished the observed hyperplasia. Adrenalectomy 3-7 days prior to initiating food restriction completely reversed both the anti-hyperplastic effect and anti-tumor promoting effect of food restriction (8). Since glucocorticoid steroids (9,10) and DHEA (11) both block the hyperplastic and tumor promoting effects of TPA, we have hypothesized that overproduction of these adrenocortical steroids in response to food restriction may mediate the tumor suppressing effect of underfeeding (12). Both DHEA and glucocorticoids inhibit oxygen free radical formation (12). Recent evidence suggests that oxygen free radicals may be widely used messengers which activate signal transduction pathways stimulating cellular proliferation (25,26). Overproduction of oxygen free radicals may also damage critical macromolecules, such as DNA, lipids and proteins, which could also enhance tumorigenesis (27,28).

Although the adrenalectomy experiments suggest an important role for the adrenal gland in mediating the tumor inhibitory effect of food restriction, more specific evidence is required to indicate a direct involvement of adrenocortical steroids. We found previously that administration of the glucocorticoid receptor antagonist mifepristone (RU-486) to CD-1 mice during an 8 day period of food restriction significantly reversed the anti-hyperplastic effect of food restriction in TPA-treated mouse epidermis (29). This effect of RU-486 suggests a direct role for elevated corticosterone levels in mediating, at least in part, the anti-hyperplastic effect of food restriction in TPA-treated mouse skin.

Plasma levels of DHEA and DHEA sulfate are much higher in the human and various primate species than in several rodent and domestic species examined (30). The level of DHEA detected by us in A/J mouse plasma is ~20% of that found in young men and women (31). DHEA sulfate levels in mouse plasma were below the sensitivity of our assay and <1/300 of the level found in young humans (31). In contrast to the consistently higher corticosterone plasma levels found by us and others in food-restricted mice, we found no change in plasma DHEA levels in food-restricted A/J mice. Thus either DHEA plays no direct role in mediating the tumor preventive and other age retarding effects of food restriction in the mouse or a congener of DHEA, with the biological properties of the native steroid, exists in the mouse and does not significantly react with the antibody for DHEA in the radioimmunoassay.

Since DHEA produces striking biological effects in laboratory rodents, many of which mimic the effects of food restriction (32), including inhibition of DMBA-induced lung tumor development in A/J mice (33), the latter possibility would seem likely. However, our experiments on DHEA plasma levels provide no direct support for the hypothesis that elevated DHEA levels in food-restricted mice contribute to the tumor inhibitory and age retarding effects of food restriction.

The results of these experiments in both skin and lung tumorigenesis models strongly suggest that secretory products of the adrenal gland play a major role in mediating the tumor inhibitory effect of food restriction in mice. The observation that administration of the glucocorticoid antagonist RU-486 to CD-1 mice significantly reverses the anti-hyperplastic effect of food restriction in TPA-treated epidermis suggests that one of the adrenal gland secretory products is corticosterone (29). The adrenocortical glucocorticoids possess marked anti-inflammatory action (34) and the inflammatory process, with its attendant infiltration of affected tissue by neutrophils and macrophages, may be an important source of oxygen free radicals. Chronic inflammation has been linked to the development of several cancers in humans, including cancer of the bladder (35), stomach (36), colon (37) and pancreas (38). There is increasing evidence that the regular use of aspirin may significantly reduce the risk of colorectal cancer (39).

Further research is needed to determine if elevated corticosterone levels unequivocally contribute to the tumor suppressing effect of food restriction in mice and what role other adrenal secretory products, such as DHEA, may have in this phenomenon.

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References

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