Potentiating effect of dietary vitamin A on photocarcinogenesis in hairless mice

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Vitamin A and its derivatives (retinoids) exert modulatory effects on epithelial differentiation and are used therapeutically against skin cancers, but the role of dietary vitamin A in ultraviolet (UV)-induced carcinogenesis is far from clear. To study this process, 220 hairless mice were given diets containing low (0.3–0.6 mg/kg; A–) or high (4–6 mg/kg; A+) amounts of retinol, which resulted after 2 months in an ~4-fold difference in liver and skin vitamin A levels as determined by HPLC. Commencing after 1 month of diet, daily irradiations with UVB (280–320 nm) or UVAB (280–380 nm) were given to 167 of the animals for 18 weeks (cumulative doses of UVB and UVA: 26 J/cm² and 168 J/cm², respectively). The first skin tumours, known to be squamous cell carcinomas, appeared after 35 weeks in the UVAB-irradiated A+ animals and 5–6 weeks later in the other groups. After one year the frequency of tumour-bearing animals was 49–63% in the A+ groups and 28–39% in the A– groups (P = 0.003). Two months later the corresponding figures were 66–72% and 50–53%, respectively (P = 0.014). Disregarding the effect of dietary vitamin A, there was no difference in the final tumour incidence between UVB- and UVAB-irradiated animals. The epidermal vitamin A content at 72 h post-irradiation was ~60% lower in A+ animals and ~10% lower in A– animals compared with the non-irradiated controls. Rather than protecting against skin cancer, a diet rich in vitamin A seems to facilitate UV carcinogenesis in hairless mice. A possible explanation is that photodecomposition of excessive vitamin A generates short-lived intermediates that may act as photosensitizers during cutaneous carcinogenesis.

Introduction

Several epidemiologic studies have given reason to regard dietary vitamin A as an important factor in the defence against cancer development (reviewed in Ref. 1), and synthetic retinoids are currently used in the treatment of cancer, including both pre-malignant and malignant tumours of the skin (2–4). This concept of a favourable effect of exogenous vitamin A is supported by findings in animal experiments that pharmacological doses of both synthetic retinoids (5,6) and natural retinyl palmitate (7) inhibit chemical carcinogenesis in the skin. On the other hand, De Luca et al. (8) recently showed that marginally vitamin A-deficient mice developed fewer skin papillomas on chemical induction than control, vitamin A-fed animals, suggesting that to some extent vitamin A is also required for tumour growth. The effects of retinoids on experimental photocarcinogenesis are more controversial (reviewed in Ref. 9). Topical retinoic acid was originally thought to promote photocarcinogenesis (10,11), but it is now frequently used to treat sun-induced epidermal dysplasia in man.

There are several reasons for implicating vitamin A in the natural defence against UV-induced skin cancer. For example, the highest level of retinol in light-protected skin is always observed in the epidermis, the outermost layer of the integument, which also contains a high proportion of retinyl esters (reviewed in Ref. 12). UV irradiation has been found to decrease the vitamin A concentration in human and murine epidermis in a dose- and wavelength-dependent manner (13–15), mainly through UV-induced destruction of retinyl esters (16). On the basis of these findings and the persistently low retinyl ester levels in UV-induced murine squamous cell carcinomas (17), we have hypothesized that the local hypovitaminosis A in irradiated skin might predispose to tumour formation (18).

In the present study we addressed the question as to whether dietary vitamin A supplementation would mitigate UV-induced hypovitaminosis A in hairless mouse skin and, as a consequence, could influence the animals’ susceptibility to photocarcinogenesis. Much to our surprise, we found that vitamin A supplementation enhanced photocarcinogenesis, an observation that has led to new hypotheses about the interaction between vitamin A and UV radiation in the skin.

Materials and methods

Diet tolerance study

The relationship between retinol intake and vitamin A status was studied in 41 hairless female mice (hr/h) obtained from Bomholtsgård Breeding and Research Centre (Ry, Denmark) at the age of 6 weeks. Eight animals were killed on arrival and served as controls. The remaining animals were divided into three equal-sized groups, each of which received one of the following semi-synthetic diets (Chemovit, Hellerup, Denmark), differing only in the retinol (Davitin A 500) supplementation: A– (0.6 mg retinol/kg), A+ (6 mg/kg) and A++ (60 mg/kg). The vitamin A content of the food mixtures was assessed by HPLC (19). The animals had free access to food and were kept at room temperature in a day-light-simulated room. After 2–4 months animals were killed and samples of blood, liver and heat-separated dorsal epidermis were collected. The samples were stored at −70°C until analysed within some months by HPLC of saponified extracts (19,20). The epidermal retinol concentrations were related to the protein content of the samples determined by a biuret technique (19).

Carcinogenesis study

A total of 220 lightly pigmented hairless female mice of the Oslo/Bom inbred strain (Bomholtsgård) were divided into six groups. Two groups, consisting of 22 animals each, served as controls and were not irradiated. Four groups, each consisting of 44 mice, were treated with various UV regimens with or without vitamin A supplementation (see below). The mice had free access to water and food. One half of the animals were fed a standard laboratory diet enriched with vitamin A (A+; see above), while the other half were given an unsupplemented diet (A–) from the same manufacturer. The mice were fed with the diets for 1 month before the irradiations began. Owing to problems with food delivery, a new manufacturer (Altromin, Lage, Germany) was contracted during the study. The new pellets (C1000 and C1016) contained a fixed formula of corn meal, oat fibres and milk powder, together with standard additions of essential fatty acids and vitamins, with or...
without retinol supplementation selected to approximate those of the previous A- and A+ diets. The retinol content was checked by HPLC (see Results). The new pellets were introduced simultaneously to all groups 2 months into the study.

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Diet group | Serum µg/dl | Liver µg/g ww | Epidermis ng/mg protein
--- | --- | --- | ---
Before (baseline; n = 8) | 49.1 ± 9.5 | 500 ± 145 | 135.5 ± 7.9
A- (0.6 mg/kg; 2 months) | 45.0 ± 12.7 | 389 ± 29 | 8.8 ± 2.7
(0.6 mg/kg; 4 months) | 38.2 ± 8.9 | 441 ± 64 | 7.5 ± 2.7
A+ (6 mg/kg; 2 months) | 51.0 ± 21.5 | 2040 ± 187 | 32.0 ± 9.6
A+++ (60 mg/kg; 2 months) | 112.0 ± 33.3 | 10 951 ± 2223 | 101.4 ± 14.7

For experimental details see Materials and methods.

Table I. Diet tolerance study. Effects of three different levels of retinol supplementation on serum, liver and epidermal concentrations (mean ± SD) of vitamin A in non-irradiated mice (n = 4, except in the ‘before’ group)

Effects of vitamin A supplementation on UV-carcinogenesis

A total of 220 animals were fed A- or A+ diets, and 176 of these were then irradiated with either UVB or UVAB for 18 weeks and observed for up to 60 weeks for development of skin tumours (Table II). The UV-exposed animals (groups 1–4) developed a slight transient erythema, followed in some cases by hyperpigmentation, and showed a high incidence (28–63%) of skin tumours after 1 year. As shown in Figure 1, the first tumours, known to be squamous cell carcinomas (21), appeared in the UVAB-treated A+ mice, and the cumulative incidence of tumours was significantly higher in the vitamin A-supplemented groups than in the unsupplemented groups both after 1 year (P = 0.003; see also Table II) and after 60 weeks (P = 0.014). Disregarding the effects of vitamin A supplementation, there were no differences in the final tumour incidence between animals treated with UVAB (groups 1 and 2) and UVB (groups 3 and 4), but tumours tended to appear earlier in the two former groups, which may partially be caused by the slightly higher UVB dose (30 vs 26 J/cm²) in the UVAB-irradiated animals (see Discussion). All animals appeared to thrive until they were killed when tumour growth became evident. The non-irradiated controls (groups 5 and 6) remained healthy and developed no tumours during the study period.

Epidermal vitamin A levels in relation to type of diet and irradiation

Table III shows the retinol concentrations in saponified skin obtained from A- and A+ animals at 72 h after the last irradiation 2 months into the study. In comparison with non-irradiated controls, UVB- and UVAB-irradiated animals showed either marginally (minus 9–11%; A- groups) or markedly (minus 56–57%; A+ groups) lower retinol values. As a consequence, the 4-fold difference in epidermal retinol between non-irradiated A- and A+ animals was reduced to 2-fold after irradiation.

Discussion

The results of our study were unexpected, for two reasons. First, the hr/h and Oslo/Bom strains of hairless mice were more sensitive to vitamin A supplementation than was anticipated from data in the literature (22). Addition of no more than 4–6 mg of retinol per kg diet, which should correspond to a daily intake of ~30 µg (100 IU) per animal (7,23), produced steady-state concentrations of vitamin A in the liver and skin that were well above the normal range (20), and 10 times this amount of supplementation elicited overt signs of hypervitaminosis A. Secondly, retinol-supplemented animals showed an increased incidence of UV-induced skin tumours compared with unsupplemented controls. This result, which was obtained with both UVB and UVAB irradiation, differs from the inability of dietary vitamin A to influence UV carcinogenesis observed in a study by Kelly et al. (23). However, they used a smaller number of animals of a different strain (Skh-hr), a different dose regime (oral gavage of 60 or 300 IU of retinyl palmitate thrice weekly), and a different UV protocol (sub-erythemogenic doses 2–5 times per week for 25 weeks), which may explain the discrepancy between the results. Clearly, however, both studies show that vitamin A supplementation offers no protection against murine photocarcinogenesis.

The lack of protection against UV-induced skin tumours is
at variance with the inhibitory effects of high doses of oral retinoic acid on chemically induced skin tumours reported by some (6), but not all investigators (24). However, vitamin A is only slowly metabolized to retinoic acid, which probably exerts its anti-cancer effects via binding to the nuclear retinoid receptors (25). Moreover, most positive studies in mouse skin have used cancer induction protocols yielding predominantly tumours with mutations in the Ha-ras oncogene as the initiating event (26), a situation that is frequently associated with loss of retinoic acid receptors (27). Ras mutations are not so frequent in UV-induced tumours and this could be one reason for the failure of vitamin A to inhibit photocarcinogenesis.

The reason for a possible stimulatory effect of vitamin A on photocarcinogenesis is more difficult to explain. In analogy with the findings in chemically-induced skin cancers (8), it could be argued that alleviation of a pre-existing vitamin A deficiency might stimulate keratinocyte proliferation in general and hence also increase tumour growth. However, our A− animals were clearly not deficient to the extent that keratinocyte proliferation would have been suppressed. In comparison with many other species, murine skin normally contains abundant vitamin A (28), and although our non-supplemented animals eventually showed somewhat reduced retinol concentrations in their epidermis, the values still exceeded those seen, for example in normal human skin (~1 ng/mg protein). Furthermore, although epidermal vitamin A is acutely decreased after irradiation (15), compensatory mechanisms leading to a rectification within 2–3 days are normally elicited (15). Thus our A− animals were found to have practically normalized their epidermal vitamin A level within 72 h post-irradiation. In contrast, the A+ animals only regained ~40% of their pre-irradiation epidermal value of vitamin A in the same period of time (see Table III), probably illustrating the fact that homeostasis will only sustain an adequate, but not a superfluous, level of vitamin A in the skin. Taken together, our results suggest that a prolonged depletion of vitamin A is unlikely to occur after long-term UV irradiation and is probably not a direct cause of the low retinyl ester levels previously observed in UV-induced squamous cell carcinomas (17,29,30).

It is clear that new explanations for the stimulatory effect of vitamin A on photocarcinogenesis must be sought. We favour a hypothesis incriminating the increased production of free radicals in irradiated skin. It has previously been shown that UV irradiation of retinyl acetate in methanol produces several short-lived intermediates and free radicals, some of which are potential photosensitizers (31). Provided that similar reactions occur in irradiated skin, an excessive deposition of vitamin A in the epidermis might lead to drastically increased levels of free radicals during irradiation and hence to stimulated carcinogenesis. In fact, this hypothesis even makes it possible to explain the earlier appearance of skin tumours in UV AB-irradiated as compared with UVB-irradiated A+ animals (Figure 1). It has previously been shown that addition of high doses of UVA to UVB will markedly enhance the photodestruction of epidermal vitamin A, for the simple reason that the absorption spectrum of retinol extends far into the UV A region (13). Speculatively, if animals are also fed high doses of vitamin A the associated increase in free radicals will become large enough to enhance tumorigenesis. If this

![Fig. 1. Cumulative incidence of UV-induced skin cancer (% of tumor-bearing animals) in the various treatment groups. For details of the treatments, see Table II. The results in groups 5 and 6 (no tumors appearing during the whole experiment) are not shown.](image)

**Effect of vitamin A on photocarcinogenesis**

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<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Vitamin A supplementation</th>
<th>Type of irradiation</th>
<th>Total energy (J/cm²)</th>
<th>Tumor incidence after 1 year (%)</th>
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a As discussed under Materials and methods, the food supplier was changed during the study. As a result of this, the retinol content of the A− diet varied from 0.4–0.6 mg/kg (mean 0.5) and that of the A+ diet from 4–6 mg/kg (mean 5.0).

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Table III. Carcinogenesis study. Epidermal vitamin A concentrations (ng/mg protein) in dorsal skin from irradiated and non-irradiated mice fed different levels of retinol for 2 months (mean ± SD; n = 4).

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Table II. Carcinogenesis study. Some characteristics of the experimental groups.

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For an explanation see Table II.

1 The animals received 1 month of irradiation. To avoid immediate effects of UV radiation, skin samples were routinely collected 72 h post-irradiation. For experimental details, see Materials and methods.

2 For an explanation see Table II.
hypothesis is correct, a combination of high-dose UVA exposure and excessive vitamin A intake must be regarded as a separate risk factor in UVB-induced skin carcinogenesis. In this context it is interesting to note that Lo et al. (31) found that retinoic acid is less prone than vitamin A to produce primary intermediates, suggesting that therapy with the former compound is less likely to induce skin photosensitivity.

In conclusion and contrary to our original belief, an abundance of vitamin A in the epidermis seems to be a foe instead of a friend in UV-exposed mouse skin, at least with respect to tumour development. Although we do not know if this effect also applies to the human situation, our study undoubtedly adds to the list of negative reports concerning dietary vitamin A intervention and prevention of cancer (32,33). The possibility that vitamin A-derived free radicals are generated in the skin during UVA irradiation and contribute to the genomic and other effects of sun damage needs to be further investigated.

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References


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