

Beta-carotene in Prevention of Cutaneous Carcinogenesis

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Beta-carotene, administered orally to mice, caused a decrease in angiogenesis evoked by HPV-transformed tumorigenic cell lines (SKv-t, HeLa). It did not affect angiogenesis induced by the non-tumorigenic SKv (not-t) cell line, and increased lymphocyte-induced immune angiogenesis. We suggest that the anti-cancerogenic effect of beta-carotene may be due, at least in part, to its inhibitory effect on formation of new blood vessels within the tumour mass. Key words: Cell-induced angiogenesis; Immune angiogenesis.

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Beta-carotene is known as an ubiquitous free radical quencher (1, 2, 3). It has been proved effective in prevention of skin tumours (4, 5) and other cancers (6, 7). It was presumed to affect the promotional phase of carcinogenesis (5), but its exact mechanism

of action in preventing cancer development remains unknown.

A method for the evaluation of anticancerogenic potential of chemical compounds is the tumour cell-induced angiogenesis (TIA) assay (2, 8, 9). Tumour cells are capable of inducing angiogenesis when injected intradermally into host that is immunosuppressed and unable to reject the graft of foreign cells. Injected cells produce angiogenic factors that stimulate host dermal blood vessels to proliferate around the site of injection.

Lymphocytes injected intradermally into an immunosuppressed host also induce angiogenesis. This assay serves to measure the immunocompetence of cells (10).

In our previous studies we have shown that various retinoids inhibit, to varying degrees, angiogenesis induced by transformed cells and that they modulate lymphocyte-induced angiogenesis (LIA) in mice (11, 12)

The aim of the present study was to evaluate whether beta-carotene can influence angiogenesis

Table I. Newly formed blood vessels (mean \pm SD) at injection site in mice treated with 0.25% beta-carotene or 0.25% placebo solution

No. of mice	injected cells	placebo	N	beta-carotene	N
12	normal human lymphocyte	10.2 \pm 2.3	23	13.5 \pm 2.4*	27
12	SKV non-t	12.9 \pm 2.7	11	12.1 \pm 1.6	12
12	SKv	15.1 \pm 2.4	24	12.1 \pm 1.6*	14
12	HeLa	15.1 \pm 2.0	8	10.8 \pm 3.8*	11

N number of injection sites evaluated for angiogenic response.

* significant differences at at least $p < 0.05$.

induced by epidermal tumour cell lines and to assess, by means of the LIA assay, whether beta-carotene is capable of affecting the immune response.

MATERIAL AND METHODS

Mice

Sixty-four 3-week-old Skh-HR1 hairless mice were used in the study. Thirty-two mice were fed with an 0.25% solution of beta-carotene in water and 32 mice were given 0.25% water placebo solution (both supplied by Hoffmann-La-Roche, Switzerland). These solutions were given ad libitum to the mice, instead of drinking water (approximately 5 ml daily). To check beta-carotene bioavailability, serum levels of beta-carotene were assessed in 8 mice from each group by means of high-performance liquid chromatography, *ad modum* Nierenberg (13). The animals were used as recipients in angiogenesis assays after 2 months of receiving beta-carotene.

Cells

Four cell types were used: 1) normal human lymphocytes obtained from healthy volunteers, 2) HeLa cell cultures established from cervical cancer, bearing the HPV 18 genome (American Tissue Type Culture Collection), 3) SKv non-tumorigenic, and 4) SKv tumorigenic keratinocytes, both established from Bowenoid papulosis associated with HPV-16 (14).

Angiogenesis assay

Two hours before the assay, mice were X-irradiated with 600 R, then anesthetized with 3.6% chloral hydrate. Each mouse received cell suspensions intradermally to evoke angiogenesis (8 injections per mouse). After 3 days, mice were killed and the inner surface of their skin dissected. The number of newly formed blood vessels growing into injection sites was counted under the dissecting microscope according to criteria described by Sidky & Auerbach (10).

The quantity of cells used for injections was calculated according to experiments in which different numbers of cells of each cell line were examined for their angiogenic capability. Angiogenesis increased linearly with the greater number of injected cells and depended on the type of injected cell line, which is in agreement with the findings of other workers (8, 10). In order to obtain angiogenesis of the same order (best visualization at about 10–15 newly

formed blood vessels per injection site), various quantities of cells were used for various cell lines. HeLa cells were injected, numbering 10^5 cells per injection site. SKv non-t and SKvt cells were used, numbering 2×10^5 . Normal human lymphocytes giving weak angiogenic response were injected intradermally, numbering 10^6 cells per injection site. All cells were suspended in 0.1 ml of PBS.

RESULTS

Results (summarized in Table I) show that oral beta-carotene administration reduces tumour-induced angiogenesis (TIA) evoked by both HeLa and SKv-t cell lines. No difference between beta-carotene and placebo fed mice was observed when a non-t SKv cell line was used for induction of angiogenesis. LIA was increased in beta-carotene fed mice, as compared with the control group.

All differences were statistically significant at least $p < 0.05$, as tested by Students *t*-test.

Serum levels of beta-carotene assessed after 2 weeks of feeding were found to be 35.5 ± 2.04 μ m/ml (mean \pm SD). In control mice, no beta-carotene was detected.

DISCUSSION

The study showed that beta-carotene is capable of reducing angiogenesis evoked by the tumorigenic cell lines: HeLa associated with HPV18 genome, and HPV16 associated SKv-t cells, but has no effect on angiogenesis induced by SKv non-t cells. Both SKv cell lines are of the same origin and contain numerous copies of HPV-16, but differ in their ability (SKv-t) or lack of ability (SKv non-t) to form tumours with characteristics of Bowen's atypia in nude mice and immunosuppressed hamsters (14). This decrease occurs when cell lines with very high angiogenic capability (HeLa and SKv-t cell) are used but does not occur in comparison with a baseline for the other types of cells.

Angiogenesis induced by normal human lymphocytes was shown to be increased even in mice treated for a prolonged time with beta-carotene. These results indicate that inhibition of tumour cell induced blood vessel formation and stimulation of immune response may be two different pathways by which beta-carotene or its metabolites exhibit their anti-carcinogenic action.

The modulatory effect of beta-carotene on TIA and LIA is comparable to that found in our previous studies for certain retinoids (etretinate and acitretin) (12 and unpublished data).

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