Inhibition of Tumorigenesis by Topical Application of Low Doses of Vitamin A Acid and Fluorouracil

In a previous study where vitamin A acid in a high dose and concentration was used simultaneously with dimethylbenzanthracene on rabbit's skin, the yield of tumors was markedly increased[1]. In a further study, using vitamin A acid and fluorouracil in combination on the keratoacanthoma, tumor regression was markedly enhanced[2]. The purpose of the present study was to observe both the effect of a low concentration of vitamin A acid on tumorigenesis as well as the effect of vitamin A acid used in combination with fluorouracil on tumorigenesis.

Materials and methods. Thirty five male albino rabbits (average wt. 1 kg) had the inner surface of their right ear auricles painted twice weekly with 1% 7,12-dimethylbenzanthracene (DMBA) in lanolin. After 3 weeks of DMBA applications, the rabbits had enlarged hair follicle orifices with some rabbits having a few tiny tumors. This suggested that the rabbits, with additional paintings, would be apt to develop tumors. The animals were divided into 7 groups, 5 animals per group and were subject to the drug combination, dosages and schedules outlined in the Table. The corresponding left ear auricles of all the rabbits served as controls.

Biopsies taken from the control ears as well as from the DMBA treated ears were sliced into 1 mm^3 pieces and placed in 4% glutaraldehyde in phosphate buffer for 1.5 h, followed by fixation in 2% osmium tetroxide buffered to pH 7.4. The tissues were dehydrated in graded strengths of ethanol and embedded in Epon. Sections 1–2 μm thick and ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop 1A electron microscope.

Results. Gross results. Rabbit ears treated with DMBA for 3 weeks had prominent follicular ostea. If no further DMBA was applied, rabbits had 3–4 tumors per ear 3 weeks latter. Those continuing to receive 3 additional weeks of applications of DMBA had 6–7 tumors per ear. When the sites on the ears were treated for 3 weeks with DMBA and then subject to vitamin A acid and 5-FU, the tumor yield was decreased when examined on the 6th week. Vitamin A acid applications with continued applications of DMBA resulted in a yield of 3–4 tumors per ear on examination by the 6th week. Fluorouracil applications with DMBA did not have an effect on reducing the tumor yield. It is interesting to note that even with the continued use of DMBA applied with vitamin A or fluorouracil (Experiments 6 and 7 Table), the tumor yield was never higher than using DMBA alone. DMBA applied with vitamin A and 5-FU (Experiment 8, Table) resulted in a yield of 2–3 tumors per rabbit ear.

A protocol of the experimental materials, methods and results

Experiment	Rabbit ears treated only with DMBA for 3 weeks, followed by applications of	No. of tumors per animal at 6 weeks
1	No. further treatment	3–4
2[a]	0.1% vitaminA[b]	2–3
3	2% 5-FU	3–4
4	0.1% vitamin A and 2% 5-FU	2–3
5	1% DMBA	6–7
6	0.1% vitamin A and 1% DMBA	3–4
7	2% 5-FU and 1% DMBA	6–7
8	0.1% vitamin A and 2% 5-FU and DMBA	2–3

[a] Experiments 2–8 had 5 animals per experiment and were treated for 3 days/week for 3 additional weeks giving 6 weeks of total treatment. Biopsies were taken 5 days after last drug application.

[b] Each application of vitamin A and/or fluorouracil (averaging 0.25 g) was applied by means of a wooden stick spatula.

Microscopic results. When vitamin A acid alone was applied to epithelium pretreated with DMBA, a slight increase in the rough-surfaced endoplasmic reticulum as well as the Golgi apparatus was observed along with the presence of mucigen droplets in the cytoplasm. Keratinocytes treated with DMBA followed by 5-FU applications produced cytoplasmic vacuoles and few pycnotic nuclei. The most striking observation when vitamin A acid and 5-FU are applied is a reduction of heterochromatin in the nuclei. When DMBA is applied with vitamin A acid and 5-FU, an almost complete loss of heterochromatin in the nuclei of the keratinocytes results.

Discussion. Vitamin A produces extremely varied responses in cell differentiation and epithelial tumorigenesis. Vitamin A palmitate in the hamster cheek pouch induced several more tumors than using DMBA alone[3,4]. In a previous investigation, a high dose (3%) of vitamin A acid produced an increase yield of keratoacanthomas in the rabbit[1].

In contrast to the above findings, SHAMBERGER[5] has reported on the marked reduction in tumor incidence with retinyl acetate. BOLLAG[6] has shown that vitamin A acid had no inhibitory effect on the growth of transplantable tumors but had a therapeutic effect on established skin tumors induced by DMBA. We have reported the increased therapeutic effect of vitamin A acid on rabbit keratoacanthoma[7]. LOGAN[8] has noted that the mechanism of action of vitamin A appears to be dose dependent in experimental situations.

Even with the continued use of DMBA in this study, vitamin A and 5-FU inhibit tumorigenesis. The most striking cytologic observation is a marked reduction in heterochromatin in the nuclei of the keratinocytes. Vitamin A and 5-FU may work synergistically to uncoil the coiled chronema which results in tumor inhibition or perhaps vitamin A and/or 5-FU complex with DMBA in the vehicle and inhibit the penetration or activity of DMBA[9].

Zusammenfassung. Reihenuntersuchung zur Prüfung der Beeinflussung der Tumorgenese beim Kaninchen durch Vitamin A und Fluorouracil. Mit Hautbepinselung von Dimethylbenzanthracen erzeugte Tumoren werden sowohl durch Vitamin A wie durch die Kombination Vitamin A + 5-FU in Wachstum und Neubildung beeinflusst.

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