Prophylaxis of Chemically Induced Papillomas and Carcinomas of Mouse Skin by Vitamin A-Acid

Retinoic acid[1] has been shown to exert a therapeutic effect on chemically induced papillomas and carcinomas of mouse skin. These benign and malignant epithelial tumors were induced by painting dimethylbenz(α)anthracene and croton oil on mouse skin. In a therapeutic experiment systemically applied retinoic acid led to a marked regression of these tumors[2–4]. In the present investigation we were interested to find out whether retinoic acid possesses, beside its therapeutic effect, also a prophylactic effect on the induction of these papillomas and carcinomas.

Materials and methods. Female Swiss albino mice of the random-bred Füllinsdorf strain, weighing 20–22 g, were fed the mouse diet Nafag 199, containing 2500 IU of Vitamin A per kg food. Papillomas and carcinomas were induced by painting 7,12-dimethylbenz(α)anthracene (DMBA) as initiating agent and croton oil as promoting agent on the skin of mice. 150 γ of DMBA in 0.2 ml acetone were applied on days 1 and 8 on a 5 cm^2 area of shaved skin on the back of the mice. 0.5 mg of croton oil dissolved in 0.2 ml acetone were painted on the skin twice a week from day 38 on. Retinoic acid was given in the promotion phase of carcinogenesis from day 44 on until the end of the experiment, as a 1% water miscible solution, containing as solubilizers 8% Cremophor® and 10% propylene glycol. It was administered orally by stomach tube to 43 mice, in a dose of 200 mg/kg, once every 2 weeks. The control group of 44 mice received only the vehicle. The appearance and development of papillomas and carcinomas were recorded. The volumes of papillomas were calculated with the formula $4/3\ r^3\ \pi$, r being the mean radius. The mean number of papillomas per animal and the mean volume of papillomas per animal were determined.

Results. Papillomas. Retinoic acid has an inhibiting effect on the development of papillomas. As can be seen from the Table, on day 113 after the first treatment with DMBA, the mean number of papillomas per animal was 3.4 in the retinoic acid treated mice, whereas the control mice were bearing an average of 4.5 papillomas per animal. In the further course of the experiment the control animals showed very little change in the number of papillomas. The retinoic acid treated mice, however, showed a steady decline of their papilloma count. At the end of the experiment on day 288, the mean number of papillomas in the control group had risen insignificantly to 5.2, whereas the same value in the retinoic acid treated group had decreased significantly to 1.8. The comparison of the mean volumes of papillomas per animal shows an analogous tendency (Table). On day 113, the papillomas of the control group had reached a mean volume per animal of 62.9 mm^3, the retinoic acid treated mice only a value of 39.9 mm^3. In the next phase of the experiment the mean volume per animal in the control group rose to 519.4 mm^3 on day 288, whereas in the retinoic acid treated group this value showed a much less marked increase to only 108.0 mm^3.

Carcinomas. Retinoic acid has also a retarding influence on the induction of carcinomas. As can be seen from the Table, retinoic acid delays the appearance of carcinomas. The incidence is markedly reduced by retinoic acid. At the end of the experiment on day 288, in the control group 18 carcinomas had developed whereas in the retinoic acid treated mice only 6 had appeared.

The effect of retinoic acid is also reflected by the fact that in the control group only 16% of the animals – 4 out of 25 mice surviving day 288 – were free of papillomas and carcinomas, whereas in the retinoic acid treated group 50% – 16 out of 32 mice – had neither papillomas nor carcinomas.

Discussion. Retinoic acid, systemically applied during the promotion phase of carcinogenesis, has a definite effect on the induction of papillomas and carcinomas of mouse skin. It delays the appearance, retards the growth and induces the regression of papillomas. The delay of appearance with its reduced incidence of papillomas in the first phase of the experiment can be looked at as the manifestation of a pure prophylactic effect. On the other side, the retarded growth and the regression of papillomas in the later phase of the experiment may be considered as caused by the therapeutic effect of retinoic acid on already established papillomas, as described in previous papers[2–4]. The prophylactic effect could be demonstrated also for malignant epithelial tumors. The incidence of carcinomas was markedly reduced by retinoic acid. These results are in agreement with the findings of several authors, who described such an effect with orally administered retinol

[1] Retinoic acid = all-trans-β-retinoic acid = vitamin A-acid = Retinsäure = Tretinoin.

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Prophylaxis of epithelial tumors with retinoic acid

	Mean number of papillomas per animal				
Days after first treatment with carcinogen	0	113		211	288
Controls	0	4.5		4.5	5.2
Retinoic acid treated mice	0	3.4		2.6	1.8
	Mean volume of papillomas per animal (mm^3)				
Days after first treatment with carcinogen	0	113		211	288
Controls	0	62.9		214.3	519.4
Retinoic acid treated mice	0	39.9		56.8	108.0
	Incidence of carcinomas (cumulative number)				
Days after first treatment with carcinogen	0	113	148	176	211	288
Controls	0	0	2	8	11	18
Retinoic acid treated mice	0	0	0	2	4	6

or retinylpalmitate[5-7]. Thus retinol and retinoic acid seem to possess the same properties regarding the prophylaxis of certain epithelial tumors. Retinoic acid may be the metabolite of retinol, responsible for the latter's activity. Such a metabolic pathway of retinol has indeed been demonstrated[8,9]. Retinol would then exert its activity only after its transformation into retinoic acid. High doses of retinoic acid seem to be needed, as in a recent investigation low doses were without influence on tumor induction[10]. The mechanism of action of retinoic acid has not yet fully been elucidated. Several properties of retinoic acid may play a role in the prophylaxis and therapy of chemically induced papillomas and carcinomas. Retinoic acid may act through its effect on the growth and differentiation of epithelial tissues[11]. The prevention of metaplasia and precancerous lesions may be responsible for the lowered incidence of carcinomas. Retinoic acid may also inhibit the induction and growth of tumors by lysosomal labilization. When the lysosomal membrane is labilized[12-16], the lysosomal enzymes, released into the cytoplasm, may destroy the premalignant or the malignant cell[17]. Tumor cells could be more vulnerable than normal cells for the following reasons: The lysosomal enzymes are active in an acid milieu[18]. The tumor cell with its high anaerobic and aerobic glycolysis produces more lactic acid and has therefore a lower pH than the normal cell[19,20]. This could explain the selective sensitivity of certain tumor cells towards retinoic acid. A further hypothesis is based on the modification of the defence mechanisms. It has been shown that retinol and retinoic acid, respectively, exert an accelerating effect on graft rejection, either by an immunological or a non-immunological mechanism[21-23]. Perhaps the above-mentioned mechanisms exert a combined attack on the premalignant and malignant cell. In experimental as well as in clinical investigations the therapeutic effect of retinoic acid given either topically or systemically has been demonstrated on certain premalignant and malignant epithelial lesions[2-4,24-27]. It is rather probable, but not proved, that the mechanisms underlying the prophylactic effect on one side and the therapeutic effect on the other side are identical. It is difficult to predict whether the prophylactic effectiveness under experimental conditions has any relevance for the prophylaxis of human epithelial tumors.

Zusammenfassung. Oral verabreichte Vitamin-A-Säure besitzt bei Mäusen eine prophylaktische Wirkung auf die Entstehung von Hauptpapillomen und Hautkarzinomen, die mittels lokaler Applikation von Dimethylbenzanthracen und Krotonöl erzeugt wurden. Vitamin-A-Säure verzögert das Auftreten, verlangsamt das Wachstum und führt zur Rückbildung von Papillomen. Ferner wird die Induktion von Karzinomen gehemmt. Diese treten bei prophylaktischer Verabreichung von Vitamin-A-Säure verzögert und in deutlich verringerter Anzahl auf. Der Wirkungsmechanismus wird diskutiert.

W. BOLLAG

Department of Experimental Medicine,
F. Hoffmann-La Roche & Co. Ltd.,
CH-4002 Basel (Switzerland), 15 May 1972.

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Antigenic Variation of the Avian Myeloblastosis Virus Obtained from Chick Embryo Fibroblasts

Avian Myeloblastosis Virus (AMV) is structurally complex[1] and would be expected to contain several antigens. This virus which causes leukemia in young chickens is released from the surface of the circulating leukemic myeloblasts and is shown to contain ATPase and RNA digesting enzyme of cellular origin in its constitution which are lacking when the same virus is produced by chick embryo fibroblasts (CEF)[2]. CsCl density gradient centrifugation also revealed a difference in the density of the myeloblast and fibroblast virus of AMV[3]. It was then of interest to see whether an antigenic variation of the AMV can be obtained from myeloblast and fibroblast cells. Viruses obtained from both these cells were analyzed in an immunoelectrophoretic system with the antisera produced against Tween-Ether (TE) split viral products of myeloblast AMV, and it is shown that there is an antigenic variation in the AMV produced by myeloblast and fibroblast cell.

Myeloblast virus was recovered from the blood plasma of infected chickens diseased with myeloblastic leukemia[4]. Plasmas were pools of several birds containing about 5×10^{11} virus particles per ml as estimated by particle count[5]. Fibroblast virus was obtained from the

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