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The effects of arotinoids on rat mammary carcinogenesis

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Summary. The influence of two retinoids, Ro 13-6298, an arotinoid ethyl ester, and Ro 15-1570, an arotinoid ethyl sulfone, on rat mammary carcinogenesis was investigated. Mammary carcinomas were induced by oral administration of 15 mg dimethylbenz(a)anthracene (DMBA) to 50-day-old female Sprague-Dawley rats. Oral administration of the two retinoids significantly inhibited the development of tumors. The number and volume of mammary neoplasms were influenced in a dose-dependent manner.

Introduction

Cancer chemoprophylaxis by retinoids has been the objective of numerous investigations in mammary carcinogenesis (for reviews see [2, 10]). Obvious reasons for the study of neoplasms of rat mammary tissue are the high susceptibility of the rodent breast to chemical induction of neoplasia, the simplicity of follow-up of tumor growth, and the high incidence of mammary cancer in humans. In a preliminary study, retinyl acetate diet supplements reduced the incidence of benign mammary tumors in rats following intragastric instillation of dimethylbenz(a)anthracene (DMBA) [8]. Later it was shown that retinyl methyl ether was superior to retinyl acetate in delaying the appearance of mammary cancer [3]. *N*-(4-hydroxyphenyl) Retinamide (HPR) had an inhibitory effect on *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis [9]. The preventive effect of HPR on both DMBA-induced and MNU-induced breast cancer is enhanced by ovariectomy [6]. In addition, chronic prolactin suppression intensifies the prophylactic effect of retinyl acetate in MNU-induced rat mammary tumorigenesis [13].

The aim of this study was to investigate the influence on the development of mammary cancer of two derivatives of a new class of retinoids. These retinoids, referred to in a preliminary publication as 'arotinoids' [5], were designated 'retinoidal benzoic acid derivatives' by other workers [12]. It has been demonstrated that the arotinoid ethyl ester Ro 13-6298 in extremely low doses leads to a marked regression of skin papillomas and carcinomas in mice [1]. The arotinoid ethyl sulfone Ro 15-1570 was the first retinoid with a sulfur-containing polar end-group that exerted antineoplastic activity [4] (Fig. 1).

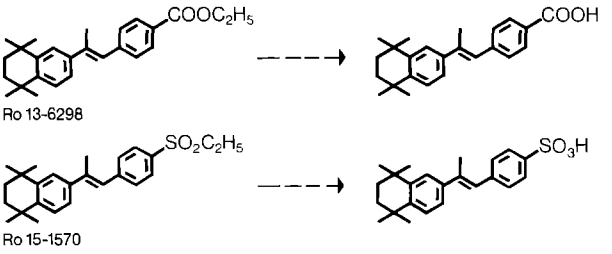

Fig. 1. Ro 13-6298, the arotinoid ethyl ester and its free acid, and Ro 15-1570, the arotinoid ethyl sulfone and the sulfonic acid analogue

Materials and methods

Female Sprague-Dawley rats from the Tierfarm Füllinsdorf Switzerland, were used. The animals were housed under temperature- and light-controlled conditions and had free access to drinking water and laboratory chow. At 50 days of age, each rat received 15 mg dimethylbenz(a)anthracene (Fluka AG, Buchs, Switzerland) dissolved in arachis oil by means of a gastric tube. Treatment began 1 day after administration of the carcinogen. Body weights were recorded and tumors were palpated and measured weekly by means of a caliper. Volumes were determined by the formula $D/2 \cdot d^2$, D and d being the larger and the smaller diameters of the tumor ellipsoid, respectively. The study was terminated after 13 weeks.

Treatment groups. The various groups were treated as detailed below.

- Control A: 74 animals received normal laboratory chow only.
- Control B: 24 animals were given 5 ml/kg arachis oil daily, 5 times weekly, in addition.
- Ro 13-6298, low dose: 8 μg/kg, dissolved in arachis oil (5 ml/kg), was given to 25 rats 5 times weekly by means of a gastric tube.
- Ro 13-6298, high dose: 25 rats received 24 μg/kg, reduced after 4 weeks to 16 μg/kg, in the same way as described above.
- Ro 15-1570, low dose: A spray-dried formulation of the following composition was prepared as a feed admix to the laboratory chow: 25% Ro 15-1570, 25% gelatine, 50% sucrose. This was given to 45 animals in a dose of 0.9 mg/kg per day each.

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– Ro 15-1570, high dose: 3.0 mg/kg, reduced after 4 weeks to 2.2 mg/kg, of the same spray-dried formulation was given to 45 rats in the diet.

Results

DMBA had induced mammary tumors in 96% of the control rats at the end of the study. The percentage of tumor-bearing animals in the two low-dose-treated groups was not significantly different from the control value, whereas this percentage was reduced by the high doses of Ro 13-6298 and Ro 15-1570 to 72% and 73%, respectively. The first tumors appeared in controls and retinoid-treated groups 6 weeks after DMBA dosing. On termination of the study the low dose of Ro 13-6298 had reduced the number of tumors per animal by 31%, and the high dose had achieved a reduction by 48%. Ro 15-1570 reduced the tumor number by 27% and 53%, respectively, compared with controls (Table 1). A growth curve indicating tumor volume per animal is shown in Fig. 2. The tumor growth reduction was significant at $P<0.05$ after 10 weeks in the low-dose groups, after 7 weeks with the high dose of Ro 13-6298, and after 9 weeks with the high dose of Ro 15-1570.

Body weight gain was significantly retarded only in the high-dose groups: by 23% with Ro 13-6298, and by 6.3% with Ro 15-1570, compared with controls. Rats treated with high doses of either retinoid showed slight to moderate signs of hypervitaminosis A (reduced weight gain, epistaxis, hair loss, desquamation).

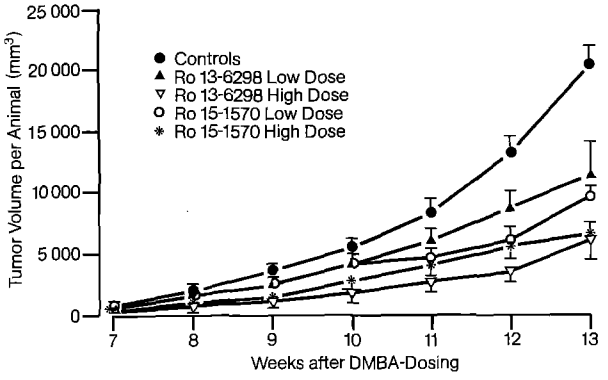

Fig. 2. Mean mammary tumor volume in cubic millimeters per rat from week 7 to week 13

Discussion

In the present study, DMBA-induced mammary carcinogenesis was markedly inhibited by oral administration of two types of arotinoids. The mean number of tumors and the total tumor volume per animal were reduced in a dose-dependent manner in rats treated with these retinoids However, first tumor appearance was not delayed, and the incidence of tumor-bearing animals was only moderately decreased by this treatment. This may be explained by the high early tumor yield resulting from the high dose of 15 mg DMBA per rat used. A low dose of DMBA might probably have delayed first tumor appearance [8]. As regards the tolerance of the retinoid treatment, hypervitaminosis A occurred in the rats treated with the two arotinoids, but the symptoms disappeared after dose adaptation.

The mode of action by which retinoids inhibit carcinogen-induced mammary cancers is still unknown. At the molecular level it is suggested that retinoids modify gene expression in carcinogen-initiated cells [11]. It is proposed that the control of gene expression by retinoids is mediated by specific intracellular receptors. Retinoic acid-binding proteins (cRABP) have been detected in chemically induced rat mammary tumors [7]. The arotinoid ethyl ester Ro 13-6298 is metabolized to its free acid and, assuming that the ethyl sulfone is metabolized to the corresponding sulfonic acid, it can be postulated that cRABP mediates the inhibitory effects within the mammary cancer cell. Indeed, a high binding affinity of both the free carboxylic acid and the free sulfonic acid of the two arotinoids to this receptor protein has been demonstrated [4].

In conclusion, the results of this study demonstrate that the novel retinoids share the capacity to inhibit rat mammary carcinogenesis with the classical structures, e.g., retinyl acetate or retinyl methyl ether. However, when compared for toxicity and efficacy, they seem to be inferior to HPR [9].

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Table 1. Tumor number, tumor volume and body weight of rats 13 weeks after DMBA administration

	Tumor load per animal		
	Number[a]	Volume[a] (mm^3)	Body weight[a] (g)
Control A untreated	3.95 ± 0.42	20200 ± 3000	293 ± 5.0
Control B vehicle	4.15 ± 0.45	20500 ± 2600	282 ± 4.5
Ro 13-6298 8 µg/kg	2.85 ± 0.30	11500 ± 2800	256 ± 5.2
Ro 13-6298 16 µg/kg	2.15 ± 0.38	6260 ± 1620	215 ± 7.9
Ro 15-1570 0.9 mg/kg	2.90 ± 0.32	9700 ± 950	290 ± 4.9
Ro 15-1570 2.2 mg/kg	1.85 ± 0.28	6700 ± 750	275 ± 5.7

[a] Means ± SEM

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