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Inhibition of Rat Mammary Carcinogenesis by an Arotinoid without a Polar End Group (Ro 15-0778)

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Abstract—*The influence of an arotinoid without a polar end group (Ro 15-0778) on rat mammary carcinogenesis was investigated. Mammary tumors were induced by oral administration of 15 mg, 7,12-dimethylbenz-(a)anthracene (DMBA) to 50-day-old female Sprague–Dawley rats. Ro 15-0778 inhibited the development of mammary adenocarcinomas. The percentage of tumor-bearing rats, the mean number of tumors per rat as well as the mean total volume of tumors per rat were dose-dependently reduced by Ro 15-0778. The results are of particular interest, since this compound—probably because of the lack of a polar end group—does not induce the signs and symptoms of hypervitaminosis A. The inhibition of mammary cancer development by Ro 15-0778 compares favorably with that of N-(4-hydroxyphenyl) retinamide the hitherto most effective retinoid for prevention of chemically-induced mammary cancers in rats.*

INTRODUCTION

THERE is now ample evidence that retinoids possess a preventive effect on the development of chemically-induced cancer in animals [1, 2]. For a long time this research was restricted to vitamin A or natural compounds structurally related to vitamin A such as all-trans retinoic acid [3]. Prevention of cancer in man can only be accomplished by compounds with a minimum of side-effects. Therefore, the search for synthetic substances with a potentially high dissociation between cancer chemopreventive effect and toxic side effects was initiated. The first synthetic retinoid fulfilling these expectations to a certain degree was the aromatic retinoic acid analog etretinate [4]. This retinoid had an antipromoting effect, delaying or preventing the appearance of chemically-induced skin papillomas and carcinomas. In the following years, efforts were made to achieve similar results with synthetic retinoids in chemically-induced tumors of various other organs. Thus it was shown that the development of chemically-induced mammary carcinomas was delayed by a series of synthetic retinoids [5–8]. *N*-(4-hydroxyphenyl)retinamide (fenretinid) (Fig. 1A) was considered the most effective retinoid in suppressing the development of mammary cancer in rats induced either by 7,12-dimethyl-benz(a)anthracene (DMBA) or by *N*-methyl-*N*-nitrosourea (MNU) [2]. In this paper we describe the effect of an arotinoid without a polar end group (Ro 15-0778) (Fig. 1B) exerting a very marked inhibiting effect on rat mammary carcinogenesis comparable to that of fenretinid. The primary advantage of this particular arotinoid is its lack of inducing the syndrome of hypervitaminosis A [9].

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MATERIALS AND METHODS

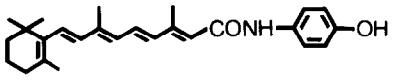
Induction of mammary cancer with DMBA

Virgin female Sprague–Dawley rats from Madörin AG, 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 7,12-dimethylbenz(a)anthracene (Fluka AG, Buchs/Switzerland) dissolved in arachis oil by means of a gastric tube.

Retinoids

The retinoid Ro 15-0778 = 1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-6-[(E)-alpha-methylstyryl]naphtalene (= A) is an arotinoid without a polar end group (Fig. 1B). Its synthesis has been described [9]. The synthesis of *N*-(4-hydroxyphenyl)retinamide (=4-HPR = fenretinid = F) (Fig. 1A) has also been described [6]. Both compounds were synthesised in the Roche Laboratories, Basle/Switzerland. They were given as feed admix. Wet-milled spray-dried formulations of A and F were mixed with the laboratory chow Kliba 343 supplied by Klingentalmühle, Basle/Switzerland.

1.A. N-(4-Hydroxyphenyl)retinamide
(= 4-HPR = Fenretinid = F)



1.B. 1,2,3,4-Tetrahydro-1,1,4,4,-tetramethyl-6-[(E)-alpha-methylstyryl] naphtalene (=Ro 15–0778 = A)

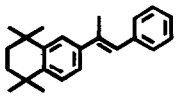


Fig. 1. Chemical structure of retinoids.

Treatment

The following treatment groups were formed:

Group 1: 30 rats, laboratory chow only, controls
Group 2: 35 rats, with feed admix of F, 90 mg/kg body wt/day
Group 3: 33 rats, with feed admix of A, 30 mg/kg body wt/day
Group 4: 36 rats, with feed admix of A, 90 mg/kg body wt/day
Group 5: 33 rats, with feed admix of A, 270 mg/kg body wt/day

In groups 2–5 feed admix was adjusted weekly according to body wt and feed consumption changes. Retinoid treatment began 1 day after carcinogen application and lasted throughout the whole experiment until the end of week 11.

Evaluation

Body wt was recorded weekly. Tumors were palpated weekly and measured by means of calipers. Volumes were calculated by the formula $\frac{D}{2} \cdot d^2$, D and d being the largest and the smallest diameter respectively, of the tumor ellipsoid. The percentage of tumor-bearing rats, the mean number of tumors per rat and the mean total volume of tumors per rat were determined.

The animals were killed at the end of week 11. All palpable nodules suspected to be mammary neoplasms were excised. One or more tissue samples from each neoplasm were fixed in 4% buffered formalin, embedded in paraffin, stained with hematoxylin–eosin and examined histopathologically.

RESULTS

Percentage of tumor-bearing rats, mean tumor number and mean total volume per rat

The arotinoid Ro 15-0778 (A) was found to have a marked influence on the development of mammary tumors induced by DMBA. In the controls the percentage of tumor-bearing rats rose to 83.9% by week 11 after the administration of a single dose of DMBA. The arotinoid A reduced dose-dependently the percentage of tumor-bearing rats to 69.7% with 30 mg/kg, to 63.9% with 90 mg/kg and to 32.5% with 270 mg/kg daily orally as feed admix. On the other side, the percentage of tumor-bearing rats in the 90 mg/kg fenretinid group was with 88.6% even higher than the controls. The same dose-dependent effect of A was evident when the mean number of mammary tumors per animal was determined. Whereas in the controls, at week 11 the number of tumors had risen to a mean of 3.65 (100%) per animal the corresponding numbers were 2.96 (81.1%) for 30 mg/kg A, 1.77 (48.5%) for 90 mg/kg A and 0.66 (18.1%) for 270 mg/kg A. The corresponding figure for 90 mg/kg fenretinid was 2.45 (67.1%). When a further parameter, the mean total tumor volume per rat, was determined, even more pronounced effects were obtained. By week 11 the controls had a mean total tumor volume per rat of 6335 mm^3 (100%), whereas the corresponding figures in the treated groups were as follows: 4904 mm^3 (77.4%) for 30 mg/kg A, 2879 mm^3 (45.4%) for 90 mg/kg A and 543 mm^3 (8.6%) for 270 mg/kg A. The rats treated with 90 mg/kg fenretinid had a mean tumor volume of 2993 mm^3 (47.2%). From Figs. 2, 3, 4 and Table 1 the time- and dose-dependency of the effects of the arotinoid (A) can be seen in comparison to the controls and to the fenretinid (F) group.

Toxicity

Body wt gain was retarded in all treated groups. Compared to controls which at week 11 had a mean body wt of 262 g, the weight of the treated groups was lower: 30 mg/kg A by 5.7%, 90 mg/kg A by 12.5%, 270 mg/kg A by 18.2% and 90 mg/kg F by 4.5%. Food consumption of the various groups was lower accordingly. Neither the arotinoid (A) nor fenretinid (F) produced any clinical signs or symptoms of the hypervitaminosis A syndrome, manifest on skin, mucous membranes (eyes, nose, mouth), central nervous system or bones.

Histopathological findings

A total of 449 subcutaneous nodules were excised. Microscopic examination revealed 443 mammary neoplasms. Except for one mammary adenoma all mammary tumors observed were adenocarcinomas. No substantial difference in the growth pattern, the mitotic activity and the differentiation of the adenocarcinomas was seen between the various groups.

DISCUSSION

In the present study it was found for the first time that the development of a chemically-induced tumor could be influenced by an arotinoid bearing at the terminal aromatic ring neither a polar end

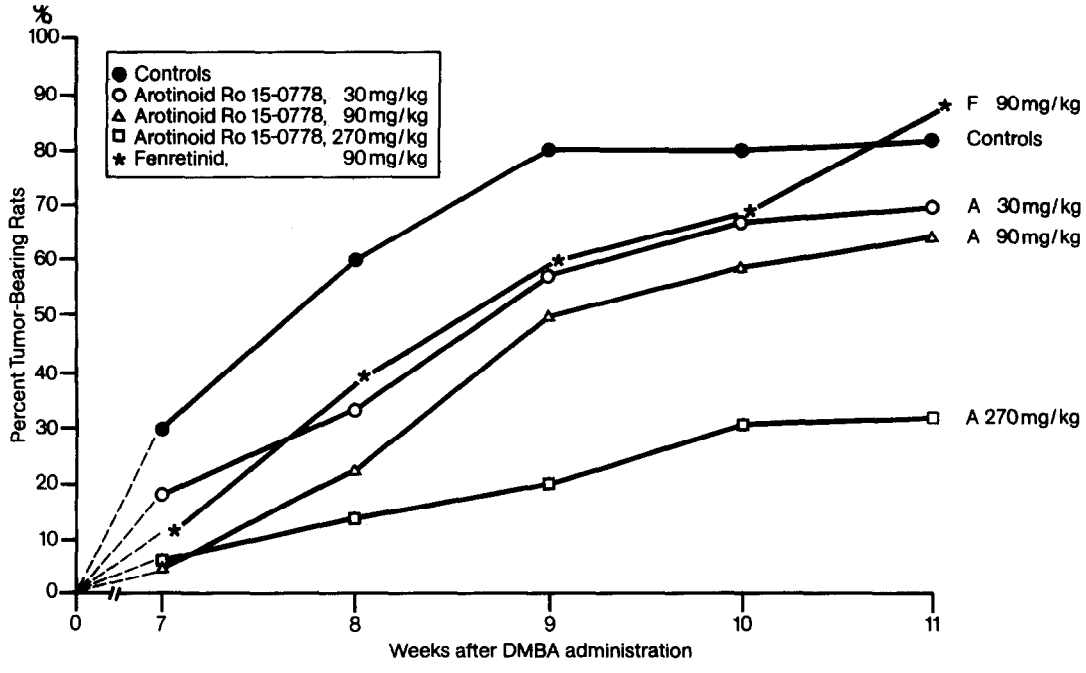


Fig. 2. Percentage of tumor-bearing animals from week 7 to week 11.

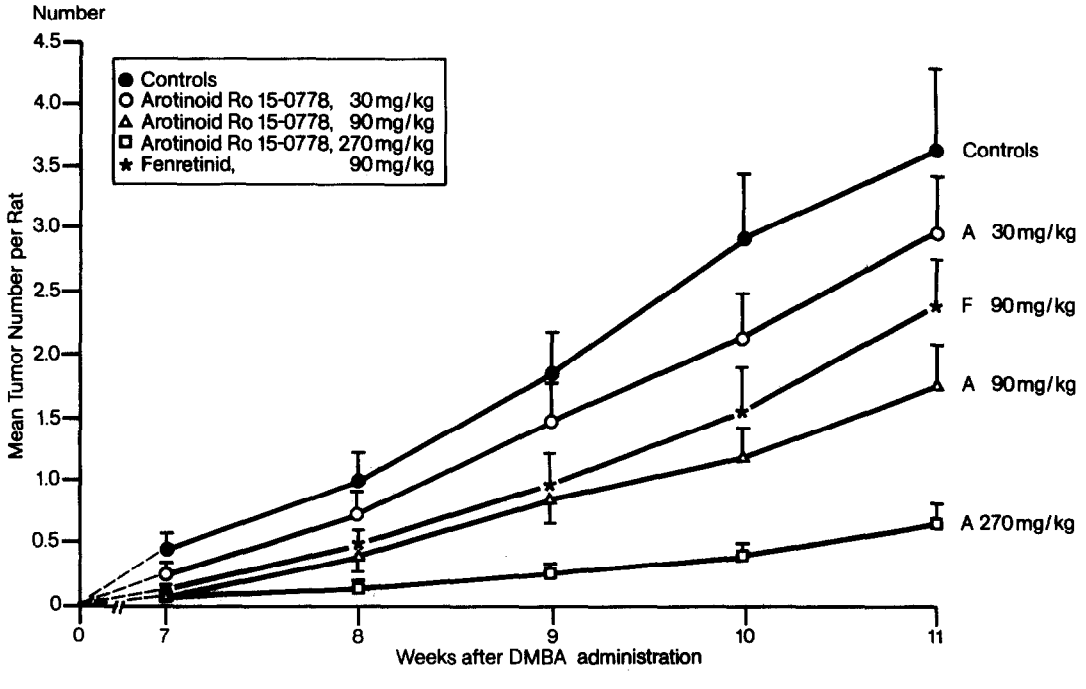


Fig. 3. Mean tumor number per animal from week 7 to week 11. Results are presented as mean values ± S.E.M.

group nor another end group which could potentially be metabolized to a benzylic alcohol or a benzoic acid. This is mechanistically of great importance in connection with the possible binding to retinol- or retinoic acid-binding protein (cRBP or cRABP).

The arotinoid Ro 15-0778 inhibits rat mammary carcinogenesis in a dose-dependent way. At a dose of 90 mg/kg/day the mean number of tumors per rat is reduced to 48.5%, and with 270 mg/kg/day to 18.1% of the controls. The mean total tumor volume per animal decreases with 90 mg/kg/day to 45.4% and with 270 mg/kg/day even to 8.6% of the controls. Compared with fenretinid, the best retinoid hitherto known for prevention of chemically-induced mammary cancer,

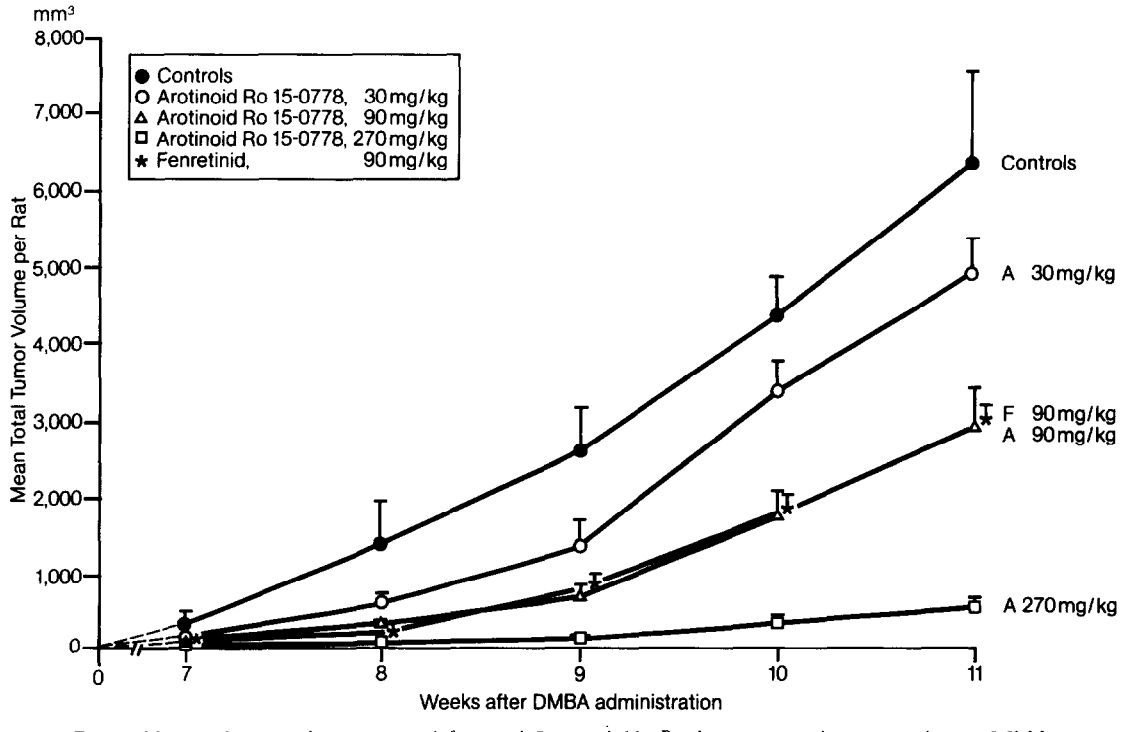


Fig. 4. Mean total tumor volume per animal from week 7 to week 11. Results are presented as mean values ± S.E.M.

Table 1. Percentage of tumor-bearing rats, mean number of tumors per rat, mean total volume of tumors per rat and mean body wt at the end of the carcinogenesis experiment at week 11 after administration of a single dose of 15 mg DMBA

	Per cent tumor-bearing rats	Mean number of tumors per rat[a,b]	Mean volume of tumors per rat[a,b] (mm^3)	Mean body weight[a,b] (g)
Control	83.9	3.65±0.66 (100%)	6335±1215 (100%)	264±4.5 (100%)
Arotinoid Ro 15-0778 (A) 30 mg/kg	69.7	2.96±0.48 (81.1%) n.s.	4904±493 (77.4%) n.s.	249±2.5 (94.3%) $P < 0.025$
Arotinoid Ro 15-0778 (A) 90 mg/kg	63.9	1.77±0.35 (48.5%) $P < 0.005$	2879±541 (45.4%) $P < 0.05$	231±2.8 (87.5%) $P < 0.0005$
Arotinoid Ro 15-0778 (A) 270 mg/kg	32.5	0.66±0.18 (18.1%) $P < 0.0005$	543±133 (8.6%) $P < 0.0025$	216±2.8 (81.8%) $P < 0.0005$
Fenretinid (F) 90 mg/kg	88.6	2.45±0.39 (67.1%) $P < 0.01$	2993±221 (47.2%) $P < 0.05$	252±3.4 (95.5%) $P < 0.025$

[a]Means ± S.E.M.
[b]*P*-values of treated groups compared to controls. Student's *t*-test

the arotinoid Ro 15-0778, has a moderately better tumor preventive activity. The slight reduction in body wt gain parallel to reduced food consumption might be caused by the impaired palatability of the feed admix. No signs or symptoms of hypervitaminosis A were observed. In this respect both compounds, fenretinid and the arotinoid Ro 15-0778, have a great advantage over other retinoids.

The activity of a retinoid in inhibiting chemically-induced mammary carcinogenesis does not imply that the same compound is also influencing tumors of other organ sites. Thus the arotinoid Ro 15-0778 e.g. does not display an effect on chemically-induced skin tumors [9]. On the other hand, the aromatic retinoid etretinate is highly active in the prevention and therapy of skin tumors [4, 10], but it has only a weak influence on the development of DMBA-induced mammary tumors (Bollag W, unpublished]. Each retinoid seems to have its own spectrum of a distinct affinity for certain tumor sites.

In spite of the immense efforts to elucidate the mechanism of action of retinoids, it is still unknown how this class of compounds acts in prevention and therapy of tumors. Much attention has been given to the role of retinoid binding proteins, particularly the cellular retinoic acid binding protein (cRABP). Many authors have discussed the possi-

bility that the effect of retinoids might be mediated by these binding proteins in an analogous way to what has been described for steroid receptors [11]. By this process they could regulate gene expression and control cell proliferation and differentiation. The arotinoid Ro 15-0778 has been examined in vitro for its affinity to cRABP. It does not bind cRABP at all [12]. These arguments cast some doubts on the importance of the role of cellular retinoid binding proteins as receptors in the mechanism of action of at least this special arotinoid.

Host and particularly hormonal factors have a marked influence on growth of carcinogen-induced rat mammary carinomas]13]. One of the most efficacious ways to affect experimentally and clinically oestrogen receptor-positive mammary tumors is by means of altering the host's oestrogenic activity. Investigations in our laboratories have shown that Ro 15-0778 has no antioestrogenic effect. This has been determined in the Allen–Doisy test (vaginal cytology) and in the uterotrophic test [Edelmann A, personal communication]. Furthermore, in the oestrogen receptor–positive human breast cancer cell line MCF 7—in contrast to the antioestrogen Tamoxifen—Ro 15-0778 did not display any growth-inhibiting activity [Hartmann D and Bollag W, unpublished]. Since DMBA-induced mammary carcinomas contain oestrogen receptors, the conclusion can be drawn that the preventive effect of Ro 15-0778 on this tumor is unlikely to be mediated by a specific antioestrogenic mechanism.

The arotinoid Ro 15-0778 may act by other mechanisms of action. Only two recently reported new fields for studies with retinoids may be mentioned. Thus retinoic acid without being bound to a retinoid binding protein has been found to inhibit the key enzyme protein kinase C probably responsible for a signal transmission to DNA replication [14]. Furthermore, in chemically-induced rat mammary tumors Ha-ras-1 oncogenes are activated [15]. It remains to be investigated whether retinoids might suppress this oncogene activation.

Concerning the clinical application of the arotinoid Ro 15-0778 for prevention of mammary cancer, this compound may have advantages over other retinoids with respect to marked efficacy and lack of side-effects. It is almost impossible to foresee the transferability of results obtained with chemically-induced mammary tumors in rats to humans. However, a clinical trial under appropriate condition may be justified.

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