Eur J Cancer Clin Oncol, Vol. 24, No. 7, pp. 1205–1209, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00

Therapeutic Effect of the Arotinoid Ro 15-0778 on Chemically Induced Rat Mammary Carcinoma

KAMPE TEELMANN* and WERNER BOLLAG

Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., CH-4002 Basle, Switzerland

Abstract—*The arotinoid Ro* 15-0778 *(temarotene) is a third generation retinoid without a polar end-group. Established, palpable and measurable rat mammary tumours, chemically induced by oral administration of* 12 mg/*animal* 7,12-*dimethylbenz*[a]*anthracene, were treated with Ro* 15-0778 *as a feed-admix in daily doses of* 100 mg/kg *for* 6 *weeks and* 200 *and* 400 mg/kg *for* 9 *weeks. For comparison, tamoxifen (anti-oestrogen) was administered as a feed-admix in doses of* 10 *and* 30 mg/kg/*day for* 9 *weeks. Treatment with Ro* 15-0778 *resulted in a marked retardation of tumour growth in the groups treated with* 100 *and* 200 mg/kg/*day and in partial or even complete tumour regression in the group receiving* 400 mg/kg/*day. Tamoxifen treatment caused a transient tumour growth inhibition during weeks* 1–5 *with subsequent re-growth of mammary tumours. Both compounds were generally well tolerated. No signs or symptoms of hypervitaminosis A were noted with Ro* 15-0778. *One single convulsive attack occurred with* 200 mg/kg/*day of Ro* 15-0778 *and* 400 mg/kg/*day caused occasional convulsions in five out of* 11 *rats. The present investigation underlines the fact that the arotinoid Ro* 15-0778 *is not only a chemopreventive agent but also exerts a chemotherapeutic effect on established, chemically induced, mammary carcinomas of the rat.*

INTRODUCTION

THE CHEMOPREVENTIVE action of various retinoids on the development of chemically induced rat mammary tumours has been repeatedly demonstrated (for reviews see [1–3]). Particularly compounds such as *N*-(4-hydroxyphenyl) retinamide (4-HPR) [4] and the arotinoid Ro 15-0778 [5] have shown marked preventive effects on rat mammary carcinogenesis. Efficacy of retinoids on established mammary cancer has been shown for 4-HPR [6], where tumours with an initial diameter of approx. 5 mm responded with complete regression in 22% of the rats.

This study was performed to investigate the *therapeutic* effect of Ro 15–0778, an arotinoid without a polar end-group, on chemically induced cancer in the rat mammary gland. For comparative purposes the anti-oestrogen tamoxifen was used.

MATERIALS AND METHODS

Induction of mammary cancer by 7,12-*dimethylbenz*-[a]*anthracene (DMBA)*

One hundred and fifty virgin female Sprague–Dawley rats obtained from the breeding farm Madörin AG, Switzerland, received by gavage a single oral dose of 12 mg DMBA (Fluka AG, Switzerland) per animal at an age of 50 days. The animals were housed under temperature- and light-controlled conditions and had free access to tap-water from drinking bottles and powdered laboratory rodent feed from glass feed hoppers. The vitamin A content of the feed was 12,000 IU/kg. Approximately 8 weeks after DMBA treatment, a sufficient number of animals had developed mammary tumours to start the therapeutic experiment.

Test compounds

The retinoid Ro 15-0778 (temarotene) = 1,2,3,4-tetrahydro-1,1,4,4,-tetramethyl-6-[(E)-alpha-methylstyryl] naphthalene (= ARO) is an arotinoid without a polar end-group. It was synthesized in the Roche laboratories, Basle, Switzerland, by Dr. Michael Klaus. The citrate salt of tamoxifen was obtained from Sigma Chemicals, St. Louis, U.S.A.

Wet-milled spray-dried formulation of Ro 15-0778 and crystalline powder of tamoxifen citrate were mixed with the laboratory chow Kliba 343 supplied by Klingentalmühle, Basle, Switzerland. Both compounds were given daily as a feed-admix.

Treatment

Sixty-six mammary tumour-bearing rats were

Accepted 26 February 1988.
*To whom correspondence and reprint requests should be addressed.

Table 1. *Tumour incidence, mean tumour number pet rat, mean tumour volume per rat and average body weight per group: before and after* 4 *and* 9 *weeks of test compound administration*

	Tumour incidence per group(%)	Mean tumour number per rat†	Mean tumour volume per rat (cm^3)†	Mean body weight per group (g)†
Before commencement of treatment				
Controls	100	3.2 ± 0.5	4.6 ± 1.0	274 ± 9.1
Arotinoid 100 mg/kg	100	3.4 ± 0.6	2.4 ± 0.5	266 ± 4.9
Arotinoid 200 mg/kg	100	2.7 ± 0.5	3.6 ± 0.1	275 ± 7.6
Arotinoid 400 mg/kg	100	3.2 ± 0.6	5.8 ± 1.9	262 ± 5.7
Tamoxifen 10 mg/kg	100	2.5 ± 0.5	2.8 ± 0.1	268 ± 4.0
Tamoxifen 30 mg/kg	100	2.3 ± 0.3	2.6 ± 0.9	262 ± 8.9
After 4 *weeks of treatment*				
Controls	100	6.0 ± 0.5	20.2 ± 4.4	287 ± 11.6
Arotinoid 100 mg/kg	100	3.6 ± 0.9*	4.5 ± 1.0**	261 ± 3.2
Arotinoid 200 mg/kg	91	3.3 ± 0.6**	1.8 ± 0.5***	232 ± 4.7***
Arotinoid 400 mg/kg	80	1.6 ± 0.6***	1.7 ± 0.8***	237 ± 7.3**
Tamoxifen 10 mg/kg	90	2.1 ± 0.5***	4.3 ± 2.2**	253 ± 6.0*
Tamoxifen 30 mg/kg	81.8	1.7 ± 0.4***	2.4 ± 1.1***	239 ± 5.5**
After 9 *weeks of treatment*				
Controls	100	6.2 ± 0.8	28.1 ± 4.8	294 ± 12.7
Arotinoid 200 mg/kg	100	3.6 ± 0.6*	4.5 ± 1.2***	272 ± 5.2
Arotinoid 400 mg/kg	70*	1.4 ± 0.5***	2.7 ± 1.6***	256 ± 5.5*
Tamoxifen 10 mg/kg	90	2.3 ± 0.5***	9.5 ± 4.7*	266 ± 6.1
Tamoxifen 30 mg/kg	90	2.1 ± 0.5***	8.3 ± 5.9*	256 ± 7.6*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; Student's *t*-test; † Means ± S.E.M.

randomly assigned to six groups consisting of 11 rats each.

Group 1: Pure laboratory chow, no admix (controls)
Group 2: Arotinoid Ro 15-0778, feed-admix, dose: 100 mg/kg/body wt/day
Group 3: Arotinoid Ro 15-0778, feed-admix, dose: 200 mg/kg/body wt/day
Group 4: Arotinoid Ro 15-0778, feed-admix, dose: 400 mg/kg/body wt/day
Group 5: Tamoxifen citrate, feed-admix, dose: 10 mg/kg/body wt/day
Group 6: Tamoxifen citrate, feed-admix, dose: 30 mg/kg/body wt/day

In groups 2–6 feed-admix was adjusted weekly according to body weight and feed consumption changes. The low arotinoid dose of 100 mg/kg/day was administered for 6 weeks; all other arotinoid and tamoxifen doses were given for 9 consecutive weeks, 7 days per week.

Evaluation

Body weight was recorded weekly. Tumours were palpated weekly and measured by means of calipers. Volumes were calculated by the formula $\frac{D}{2}.d^2$, D and d being the largest and the smallest diameter respectively, of the tumour ellipsoid. Tumour incidence per group, mean tumour number per rat and mean tumour volume per rat were determined. Tumour volume was also expressed as a percentage of baseline value.

RESULTS

Tumour incidence (*Table* 1, *Fig.* 1)

Under treatment with the arotinoid Ro 15-0778, a dose-dependent therapeutic effect on the mammary tumours was noted. A slight decrease in the percentage of tumour-bearing rats (tumour incidence) was recorded since one rat treated with 200 mg/kg/day became transiently completely tumour-free and in the 400 mg/kg/day group three rats in week 3 and four rats in week 8 showed complete regression of all tumours. In the rats treated with tamoxifen, complete tumour regression was also noted in study week 2 in three rats treated with 30 mg/kg/day, but two of these rats subsequently showed new mammary tumour formation with only one rat remaining tumour-free for the rest of the study.

Tumour number (*Table* 1, *Fig.* 2)

The mean tumour number per rat, which almost doubled, from 3.2 to 6.2 in the controls, remained almost constant in the dose groups given 100 and 200 mg/kg/day but declined from 3.2 to 1.4, by 56%, in the rats treated with the high dose of 400 mg/kg/day. A stabilization (10 mg/kg dose group) or even reduction (30 mg/kg dose group) in mammary tumour numbers was also observed in the tamoxifen-treated animals in the first study weeks, but due to new tumour formation in the second part of the study, the initial tumour count at commencement of treatment had almost been regained by the time the study was terminated.

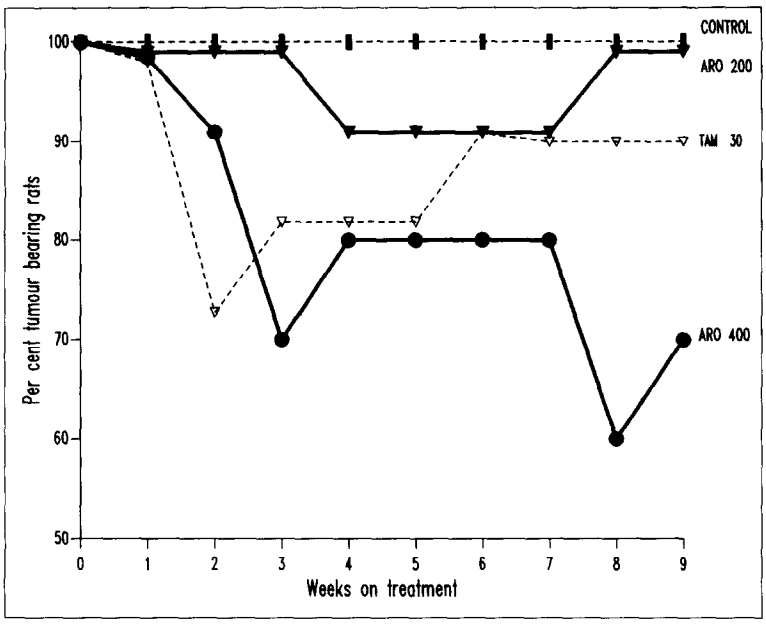

Fig. 1. Tumour incidence per group under treatment with arotinoid Ro 15-0778 (*ARO*) *in doses of* 200 *and* 400 mg/kg *body wt/day and tamoxifen* (*TAM*) 30 mg/kg *body wt/day.*

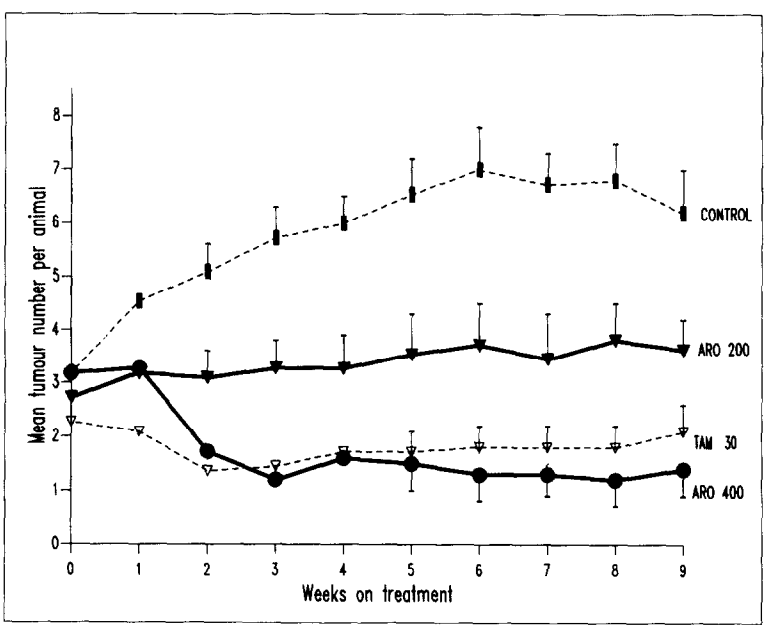

Fig. 2. Mean tumour number per animal under treatment with arotinoid Ro 15-0778 (*ARO*) *in doses of* 200 *and* 400 mg/kg *body wt/day and tamoxifen* (*TAM*) 30 mg/kg *body wt/day.*

Tumour volume (*Table* 1, *Fig.* 3)

The mean tumour volumes showed a dramatic increase from 4.6 to 28.1 cm^3, by 611%, in the untreated controls. In contrast, tumour growth was significantly inhibited by the treatment with the low dose of the arotinoid (100 mg/kg/day) and was blocked by the treatment with the intermediate arotinoid dose (200 mg/kg/day). In the high dose arotinoid group (400 mg/kg/day) tumour volumes even decreased from 5.8 to 1.7 cm^3, by 71%, in week 4, and from 5.8 to 2.7 cm^3, by 53% in week 9. This includes also the complete disappearance of a number of tumours. The effect on tumour volumes was much less pronounced in the rats treated with tamoxifen, since tumour growth was only retarded (10 mg/kg group), or transiently reduced by a maximum of 18% in week 2 (30 mg/kg group). Subsequently, under continuing tamoxifen treatment, continuous tumour re-growth occurred with final tumour volumes more than 200% higher than at start of the study.

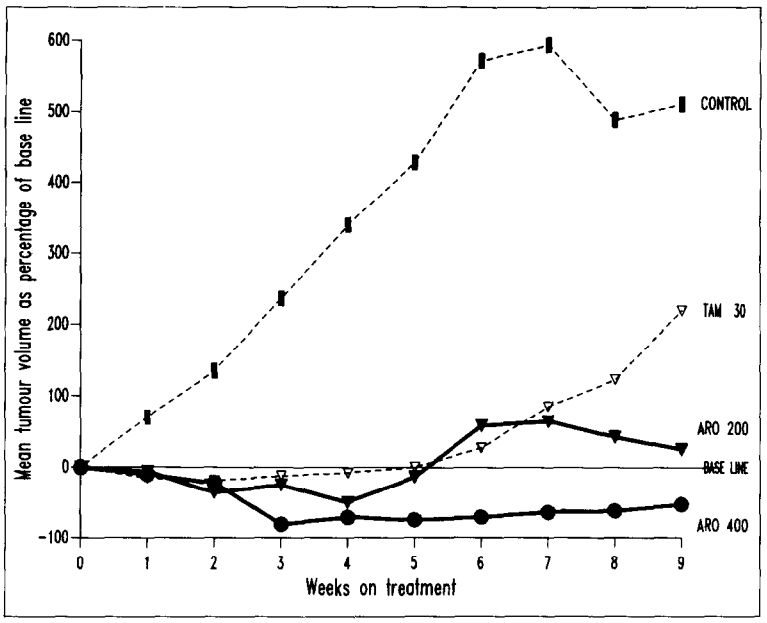

Fig. 3. Mean tumour volume per animal expressed as ± percentage of baseline values under treatment with arotinoid Ro 15-0778 *(ARO) in doses of* 200 *and* 400 mg/kg *body wt/day and tamoxifen (TAM)* 30 mg/kg *body wt/day.*

Toleration of test compounds

The mixture of the test compounds with the powdered rodent diet resulted in a dose-related reduced feed intake of more than 50%, notably in the first 2 study weeks. This reduced feed intake was accompanied by dose-related, transient weight losses of the rats given both compounds, but the weight lost was almost compensated during the second part of the study (Table 1). No signs or symptoms typical of the hypervitaminosis A syndrome [7, 8] were noted in the arotinoid-treated rats. Tamoxifen treatment was also generally well tolerated. The only adverse effects noted with Ro 15-0778 were a single event of short-lasting tonic–clonic convulsions in one rat treated with 200 mg/kg/day (week 8) and occasional convulsions in five out of 11 rats treated with 400 mg/kg/day of the arotinoid Ro15-0778 (weeks 2, 5, 7 and 9). Five rats died during the course of the study. The deaths were considered to be related to large mammary tumours and were evenly distributed through all groups including controls (no deaths were recorded in the 200 mg/kg arotinoid dose group). All rats were autopsied after termination of treatment, no test compound-related organ alterations were found.

DISCUSSION

Chemically (DMBA) induced, established mammary tumours in rats were used as targets in this study to investigate the therapeutic efficacy of the arotinoid Ro 15-0778 in comparison to the anti-oestrogen tamoxifen. The major parameters examined were retardation of tumour progression and tumour regression, respectively.

Under the conditions of this study, the arotinoid Ro 15-0778 showed anti-tumour activity in both respects: the lower doses (100 and 200 mg/kg/day) resulted in a marked retardation of tumour growth or a stabilization with unchanged tumour size; administration of the high dose (400 mg/kg/day) was associated with a marked regression of tumour volumes by 71% in week 4. Complete remission of all tumours was achieved in three out of 10 of the animals. The highest number of tumours per rat which disappeared completely were six tumours having average diameters of approximately 10 mm at commencement of treatment. There was hardly any indication of tumour re-growth during treatment with the arotinoid.

These results compared favourably with the data obtained with tamoxifen which mainly indicate retardation of tumour growth but only minor tumour regression. The tamoxifen effect was generally transient in character and lasted only for the first 4 study weeks. Thereafter, a slow but continuous increase of tumour volumes (average *ca.* 30% per week) was noted in the tamoxifen-treated rats. The reason for this progressive drug resistance is unknown.

The arotinoid Ro 15-0778 is a so-called third generation retinoid, the terminal aromatic ring of which has neither a polar end-group nor any other end-group which could potentially be metabolized to a benzylic alcohol or a benzoic acid [9]. It has been shown to be inactive against chemically induced skin papillomas of the mouse [9] and to be exceptionally well tolerated in a variety of toxicity tests on laboratory animals [8]. The short-lasting tonic–clonic convulsions observed in this study are

a new observation in vitamin A derivatives and have not been recorded with other retinoids. How far this finding must be considered species- or even strain-specific remains to be elucidated. No convulsions were noted in mice or dogs treated with similar doses for prolonged periods [8].

The inhibition of DMBA tumour induction and growth by tamoxifen is thought to be due to its competition with oestradiol in binding to the oestrogen receptor protein [10, 11]. The anti-tumour effects of tamoxifen can be reversed by progesterone [12]. Ro 15-0778 has no anti-oestrogenic effect [5] and its mode of action is still unknown. The hypothesis that the mechanism of action of retinoids in general is related to their effects on the expression of oncogenes and peptide growth factors [13] has very recently been supported by the identification of a nuclear retinoic acid receptor [14, 15] that shows homology with the steroid/thyroid receptors. Whether a retinoid without a carboxylic acid end-group, such as Ro 15-0778, can bind to the above mentioned retinoic acid receptor remains to be investigated.

In conclusion, this study underlines the fact that certain retinoids exert anti-tumour activities not only by preventing the formation of chemically induced rat mammary carcinomas, but also by exerting a therapeutic effect leading to growth retardation or partial or even complete regression of established palpable tumours. The mode of action is different from anti-oestrogenic compounds, such as tamoxifen, and is probably explained by altered gene expression. Since Ro 15-0778 lacks the side-effects of the hypervitaminosis A syndrome, a clinical trial in mammary carcinoma may be justified.

Acknowledgements—We wish to express our gratitude to Mr. C. Dobson and Dr. D. Hartmann for critically reading the manuscript. We are indebted to Mr. J. Schoerlin and Mr. V. Sutter for skilful technical assistance. We are grateful to Mrs. E. Ammari for expert secretarial work.

REFERENCES

1. Bollag W, Hartmann HR. Prevention and therapy of cancer with retinoids in animals and man. *Cancer Surveys* 1983, **2**, 293–314.
2. Bertram JS, Kolonel LN, Meyskens FL Jr. Rationale and strategies for chemoprevention of cancer in humans. *Cancer Res* 1987, **47**, 3012–3031.
3. Lippman SM, Kessler JF, Meyskens FL Jr. Retinoids as preventive and therapeutic anticancer agents (part 1). *Cancer Treat Rep* 1987, **71**, 391–405.
4. Moon RC, Thompson HJ, Becchi PJ *et al. N*-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. *Cancer Res* 1979, **39**, 1339–1346.
5. Bollag W, Hartmann HR. Inhibition of rat mammary carcinogenesis by an arotinoid without a polar end group (Ro 15-0778). *Eur J Cancer Clin Oncol* 1987, **23**, 131–135.
6. Dowlatshahi K, Mehta R, Thomas CF, Dinger NM, Moon RC. Therapeutic effect of *N*-(4-hydroxyphenyl)retinamide (4-HPR) on methylnitrosurea (MNU) induced rat mammary cancer. *Proc Am Soc Clin Oncol* 1986, **5**, 32.
7. Kamm JJ, Ashenfelter KO, Ehman CW. Preclinical and clinical toxicology of selected retinoids. In: Sporn MB, Roberts AB, Goodman DS, eds. *The Retinoids*. New York, Academic Press, 1984, Vol. 2, 287–326.
8. Teelmann K. Retinoids: toxicology and teratogenicity to date. In: MacKie RM, ed. *Pharmacology and Therapeutics*, special issue on Retinoids, 1988 (in press).
9. Loeliger P, Bollag W, Mayer H. Arotinoids, a new class of highly active retinoids. *Eur J Med Chem-Chim Ther* 1980, **15**, 9–15.
10. Jordan VC, Allen KE, Dix CJ. Pharmacology of tamoxifen on laboratory animals. *Cancer Treat Rep* 1980, **64**, 745–759.
11. Seibert K, Lippman M. Hormone receptors in breast cancer. *Clin Oncol* 1982, **1**, 735–794.
12. Robinson SP, Jordan VC. Reversal of the antitumor effects of tamoxifen by progesterone in the 7,12-dimethylbenz[*a*]anthracene-induced rat mammary carcinoma model. *Cancer Res* 1987, **47**, 5386–5390.
13. Sporn MB, Roberts AB, Roche NS, Kagechika H, Shudo K. Mechanism of action of retinoids. *J Am Acad Dermatol* 1986, **15**, 756–764.
14. Petkovich M, Brand NJ, Krust A, Chambon P. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* 1987, **330**, 444–450.
15. Giguere V, Ong ES, Segui P, Evans RM. Identification of a receptor for the morphogen retinoic acid. *Nature* 1987, **330**, 624–629.