Rat Mammary Cancer Inhibition by a Prolactin Suppressor, 2-Bromo-α-Ergokryptine (CB 154)

Evidence is accumulating that prolactin plays a major role in mammary gland carcinogenesis (for review see BOOT[1]), at least in laboratory rodents[2-6]. In these animals experimental results show that procedures which inhibit prolactin secretion will also reduce the growth of mammary tumours or cause them to regress. Certain ergot alkaloids can be used as pharmacological tools to reduce prolactin secretion. Ergocornine[7] and 2-bromo-α-ergocryptine (CB 154)[8] inhibit lactation in rats. Hormone assays have revealed that ergocornine inhibits prolactin release in rats[9], and ergocornine as well as CB 154 have been shown to reduce prolactin content of the pituitary in tumourbearing rats and mice[10,11]. Ergocornine[10-12] and CB 154[11-13] are also effective in inhibiting the growth of 7, 12-dimethyl-benz(a)anthracene (DMBA)-induced mammary tumours in rats and the development of mammary hyperplastic alveolar nodules in C3H/He mice. In these rat studies, the ergot alkaloid treatment was begun when tumours had attained a certain size. We were interested to know what effect CB 154 would have if treatment was started at the time of tumour induction.

Materials and methods. 80 female CFE (=OFA) rats (SPF, Sandoz), 8 weeks old and of ca. 180 g body weight, were used. They were given Nafag-194 pellets and water ad libitum, and were kept in rooms lighted artificially 12 h a day. They were divided into 4 groups and treated as indicated in the Table.

Mammary tumours were induced by the i.v. injection of a clear aqueous solution of 7, 12-dimethylbenz(a)anthracene (DMBA); DMBA was obtained from Th. Schuchardt (Munich). The solution had the following composition: 17.5 mg DMBA, 0.7 ml dimethyl sulfoxide (DMSO); 0.7 ml Tween 80, water ad 7.0 ml. Of this solution, the rats received 1 ml each on 2 successive days into the tail vein, resulting in a total dose of 5 mg DMBA per rat (if the injection is given slowly, 5 mg DMBA – 2 ml of the solution – can also be given in one application). The aqueous solution might be easier to inject than the recently proposed solution in DMSO alone[14].

A solution of CB 154 was prepared by adding 60 mg of tartaric acid and 1 ml of ethanol (96%) to 60 mg of CB 154, water was then added to make 100 ml. Of this solution, rats received 1 ml/100g body weight i.p. (= 6 mg CB 154 per kg) daily, except Sundays, starting on the day of the second DMBA application.

Body weights given in the Table have been obtained by subtracting the approximate tumour weight from the total weight of the rat, the approximate tumour weight (g) was taken as two thirds of the length × width × estimated depth in cm. Evaluation of tumour growth was done by multiplying 2 transverse diameters of the tumours to obtain a parameter ('area') of their size; the results of these measurements, which were taken every week, are expressed

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Group	Treatment	No. of rats	Day	Average body weight (g)	No. of rats surviving (%)	No. of rats with tumors (%)	Average tumor size per living rat ('cm²') ± S.E.M.
A	Solvent of DMBA i.v.		63	239	19	0 (0)	0
	days 0 and 1	20	119	269	19	0 (0)	0
			175	271	18	2 (11)	0.27 ± 0.22
			243	280	17 (85)	1 (6)	0.88
	DMBA i.v.		63	245	20	9 (45)	0.86 ± 0.33
B	2.5 mg/kg days 0 and 1	20	119	267	19	17 (89)	8.7 ± 2.4
			175	308	18	17 (94)	9.7 ± 2.5
			243	279	5 (25)	5 (100)	17.8 ± 5.1
C	CB 154 i.p.		63	240	20	0 (0)	0
	6 mg/kg/d days 1–80	20	119	267	19	0 (0)	0
			175	284	19	0 (0)	0
			243	313	16 (80)	0 (0)	0
D	DMBA (as B)		63	253	19	3 (16)	0.16 ± 0.09[2] (19)[a]
	+CB 154 i.p.	20	119	278	19	14 (74)	3.5 ± 1.3[3] (40)
	6 mg/kg/d		175	293	19	16 (84)	5.5 ± 1.5 (57)
	days 1–80 (8 rats)		243	326	16[1] (80)	16 (100)	13.5 ± 2.9 (76)
	days 1–100 (12 rats)						

[a] In brackets: tumor size in % of group B. [1] Chi square test: $p < 0.005$ compared to group B. [2] t-test: $p < 0.0125$ compared to group B. [3] t-test: $p < 0.05$ compared to group B.

as cm². The sum of the sizes of all tumours of each rat was used in the Table, irrespective of the number of tumours per animal. Only the data of days 63, 119, 175, and 243 are given in the Table.

The first DMBA injection was given on March 4; the experiment was terminated November 2, thus its duration was 243 days.

Results. The following points emerge, among others, from the figures in the Table. Body weight increase is not diminished by treatment with DMBA or CB 154 or both. Mortality of the DMBA treated rats during the whole experiment is considerably reduced by CB 154 ($p < 0.005$, χ^2-test, for group D, compared to group B).

Tumours were first detected at about 40 days after DMBA. The arithmetic mean of the time of appearance of the first tumour in those rats which developed tumours was 74.5 days for group B and 106.6 days for group D; this difference is statistically significant ($p < 0.01$, t-test). 50% of the DMBA-treated rats show tumours 70 days after DMBA in group B, and after 100 days in group D. All DMBA-treated rats of groups B and D surviving to the end of the experiment developed tumours. It should be noted that in group A which received only the solvent of DMBA, 2 rats developed spontaneous tumours, while there were no tumours in the animals which were treated with CB 154 for 80 days (group C).

The size of the tumours (as defined in methods) at different times is a parameter of tumour growth. The last column in the Table indicates that the mean tumour size in the CB 154-treated group is less than 50% of that of the DMBA control up to at least 119 days. The difference between the tumour sizes of the DMBA controls (group B) and that of the rats treated with CB 154 (group D) is statistically significant for day 63 ($p < 0.0125$) and for day 119 ($p < 0.05$) only. Tumours were always excized and weighed when tumour-bearing animals died during the experiment or were killed at the end of the experiment; the arithmetic mean of this weight was 101 g for group B, and 38.9 g for group D. This difference is not significant, due to the large variation of tumour weights(t-test, $0.05 < p < 0.10$).

When rats which had received 5 mg of DMBA were treated with CB 154 for days 1–80 at a dose of 1 mg/kg/day only, tumour inhibition was but minimal.

Discussion. This study demonstrates that CB 154 at a daily dose of 6 mg/kg i.p. significantly inhibits DMBA-induced tumour growth, for at least as long as the compound is given. Treatment of the rats with the ergot alkaloid was not accompanied by an inhibition of net body weight increase. Decreased food consumption will, as has been shown[15], reduce the growth of carcinogen-induced mammary tumours in rats. The observed reduction in mortality of CB 154-treated animals can probably be attributed partially to tumour inhibition since it is assumed that most of the rats in group B died from tumour growth or from infection of ulcerated tumours.

Although CB 154 treatment clearly delayed tumour appearance, reduced tumour growth, and increased survival, all the DMBA-treated animals surviving to the end of the experiment had tumours. Thus, despite beginning treatment with CB 154 24 h after the first dose of DMBA, CB 154 did not protect the animals from tumour induction. Our data are consistent with, and extend the results of HEUSON et al.[13] who in a 6 week study in animals with well developed DMBA-induced tumours showed that CB 154 increased the number of regressing tumours and reduced the number of progressing and of newly formed neoplasms. A similar inhibition of DMBA-induced mammary tumours was obtained in rats by NAGASAWA and MEITES[10] using the structurally related ergocornine.

When, in our study, treatment with CB 154 was stopped after 80 or 100 days, tumour growth inhibition ceased. This finding is consistent with the concept that CB 154 depresses mammary tumour growth by depressing reversibly prolactin secretion. In mammary tumour-bearing mice YANAI and NAGASAWA[12] found that the prolactin content of anterior pituitaries was reduced after 3 weeks daily treatment with CB 154. HEUSON et al.[13] observed that mammary tumour-bearing rats treated with CB 154 daily for 6 weeks showed a persistence of (inactive) corpora lutea, a phenomenon which is best interpreted as a functional sign of prolonged prolactin suppression[16]. CB 154 inhibits prolactin secretion selectively: STH content in anterior pituitaries of treated mice was found unchanged after prolonged treatment with the drug[11,12], and gonadotropin secretion as judged by vaginal smears was more regular in CB 154 treated tumour-bearing mice[12] and rats[13] than in untreated controls.

Using ergocornine, NAGASAWA and MEITES[10] demonstrated a 50% reduction of serum prolactin in mammary tumour-bearing rats, while PEARSON et al.[5] showed that ovariectomy plus adrenalectomy performed in similar rats reduced serum prolactin by more than 50% also, and at the same time fully repressed mammary tumours. In such rats, prolactin secretion could again be increased by perphenazine, and preliminary data suggest that this was accompanied by further tumour growth despite the absence of steroid-producing glands[5].

It should be added that CB 154 does not inhibit growth of sarcoma 37 in mice, even in sublethal doses[17]; this transplantable tumour is derived from a mammary tumour of a mouse, and is no longer hormone dependent. CB 154 also has no cytostatic effect in cell cultures; in concentrations up to 10 mg/l the drug does not inhibit multiplication of P-815 mastocytoma cells in vitro[17].

It is still uncertain as to whether prolactin is concerned in the development of human breast cancer; but it is now well established that prolactin plays an essential role in certain mammary tumours of rats and mice, that CB 154 depresses prolactic secretion and that it inhibits growth of prolactin dependent tumours. It is therefore postulated that CB 154 might be useful in the treatment of mammary cancer in man.

Zusammenfassung. 2-Br-α-Ergokryptin (=CB 154) wurde täglich während 80–100 Tagen, beginnend mit der Carcinogenapplikation, DMBA - (5 mg i.v.)-behandelten weiblichen Ratten in der Dosis 6 mg/kg i.p. verabreicht. CB 154 bewirkte ein verzögertes Erscheinen und ein langsameres Wachsen der Mamma-Tumoren sowie eine verminderte Todesrate der carcinogenbehandelten Ratten.

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