Effects of Reserpine on Development of 7,12-Dimethylbenzanthracene Induced Mammary Tumors in Female Rats[1]

Tranquilizers such as reserpine or chlorpromazine have been reported to depress hypothalamic activity, stimulate prolactin secretion and increase mammary development in rats[2]. Since prolactin is believed to be important for development of mammary tumors, it was of interest to determine the effects of reserpine on development and growth of carcinogen-induced mammary tumors (MT) in rats.

Material and methods. Virgin female Sprague-Dawley rats were housed in a temperature (75 ± 2 °F) and light (14 h/day) controlled room, and given a diet of Wayne Lab Blox (Allied Mills, Chicago, Ill.) and water ad libitum.

Treatment with reserpine before MT appearance. 76 immature female rats, each approximately 90 g in weight, were divided randomly into 3 groups and treated as follows: Group 1, intact controls, no treatment; group 2, intact and 10 µg of reserpine/100 g body wt.; group 3, intact and 100 µg of reserpine/100 g body wt. Reserpine was dissolved in saline and injected s.c. once daily (see Table I for dose schedule). At 55 days of age all rats were given a single i.v. injection of 1 ml of a lipid emulsion containing 5 mg of 7, 12-dimethylbenzanthracene (DMBA). After DMBA treatment, all animals were palpated weekly for MT. The mean latency period for MT appearance was determined by calculating the average number of days from DMBA treatment to detection of each palpable MT. All rats were sacrificed 120 days after carcinogen treatment, at which time all MT were excised and weighed. Significance of mean differences between groups was calculated by student's *t*-test.

Treatment with reserpine after MT appearance. 80 female rats were given a single injection of DMBA at 55 days of age. 75 days after DMBA treatment, when all rats weighed approximately 250 g and had at least 1 palpable MT, they were divided into 5 groups and treated as follows: Group 1, intact controls, no treatment; group 2, intact and 10 µg reserpine/100 g body wt.; group 3, ovariectomized controls, no treatment; group 4, ovariectomized and 10 µg reserpine/100 g body wt.; group 5, ovariectomized and 100 µg reserpine/100 g body wt. The lower dose of reserpine was injected s.c. once daily for 50 days; the higher dose was injected once daily for 25 days. At 0, 10, 25 and 50 days after the initial treatment, all rats were examined for number of palpable MT. 24 h after the last injection of reserpine, the rats were sacrificed and all MT were excised and weighed. Mean increase or decrease per group in number of palpable MT was determined at 0–10, 0–25 and 0–50 days after the initial reserpine treatments. Significance of differences between means was calculated by student's *t*-test.

Results. Treatment of intact rats with either 10 or 100 µg reserpine before MT appearance significantly reduced the number of MT/rat (Table I). The 100 µg dose of reserpine significantly lengthened the mean latency period for MT appearance and significantly reduced average total weight of MT/rat. The 10 µg dose of reserpine had no adverse effect on body weight gains, whereas the rats given the 100 µg dose of reserpine maintained their body weights during the treatment period but showed a marked loss (33%) in body weight by 6 weeks after termination of treatment. When these rats were sacrificed their body weights were only slightly less than the intact controls.

Treatment of intact rats with 10 µg reserpine after MT appearance significantly increased development and growth of MT (Table II). Reserpine at either dose level has no significant effect on MT development in ovariectomized rats, all rats showing marked MT regression. The ovariectomized rats treated with 100 µg reserpine lost considerable body weight after 25 days of treatment and 3 of the original 16 rats died.

Discussion and conclusions. When reserpine injections were begun before DMBA treatment, a significant decrease in incidence of MT was observed. Stimulation of rat mammary growth before carcinogen treatment by pregnancy[3], pituitary homografts[4], or hypothalamic-median eminence electrolytic lesions[5] has also been reported to significantly reduce the incidence of MT. These treatments as well as reserpine result in increased pituitary prolactin secretion and in marked mammary development[2,6]. Apparently, stimulation of rat mammary growth with development of a predominantly lobulo-alveolar system renders the mammary gland relatively refractory to the action of DMBA[3–5].

Reserpine, when administered to intact rats already bearing DMBA-induced MT, significantly stimulated development of the incipient MT. This is in accord with the concept that treatments which increase pituitary prolactin secretion result in enhanced development of

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Table I. Effect of reserpine on incidence of mammary tumors in intact rats treated with DMBA

Group and treatment[aa]	No. of rats	Rats with tumors (%)	Average No. of[dd] tumors/rat	Average total weight[dd] of tumors/rat (g)	Mean latency[dd] period (days)
1. Intact, controls	37	100	10.9 ± 0.7[a]	12.2 ± 2.3[d]	70.2 ± 2.0[g]
2. Intact, reserpine, 10 µg[bb]	18	100	7.5 ± 1.2[b]	8.5 ± 2.2[e]	68.0 ± 3.2[h]
3. Intact, reserpine, 100 µg[cc]	21	81	3.3 ± 0.7[c]	2.6 ± 1.4[f]	98.1 ± 4.4[i]

[aa] DMBA was administered when the rats were 55 days old. [bb] Reserpine (10 µg/100 g/body wt.) was injected once daily beginning at age 30 days and for 40 days thereafter. [cc] Reserpine (100 µg/100 g/body wt.) was injected once daily beginning at age 50 days and for 15 days thereafter. [dd] Mean ± S.E. [a,c], [g,i], [h,i] = P 0.001. [b,c] = P 0.005. [a,b] = P 0.02. [d,f], [e,f] = P 0.05.

Table II. Effects of reserpine on growth and development of DMBA induced mammary tumors in female rats

Group and treatment[aa]	Total No. of rats	Average No. of palpable tumors/rat					Average total weight of tumors/rat (g)
		Initial	10 days after treatment	25 days after treatment	50 days after treatment	Change (%)	
1. Controls, intact	16	3.3 ± 0.5[bb]	3.9 ± 0.6[a]	5.3 ± 0.8[d]	6.9 ± 1.0[g]	+109	12.0 ± 3.8[j]
2. Reserpine (10 μg), intact	16	3.3 ± 0.7	5.2 ± 1.1[b]	7.1 ± 1.3[e]	10.6 ± 1.8[h]	+221	15.5 ± 3.4
3. Controls, ovariectomized	19	4.1 ± 0.6	2.6 ± 0.5[c]	1.4 ± 0.4[f]	1.3 ± 0.4[i]	− 68	1.9 ± 1.2[k]
4. Reserpine (10 μg), ovariectomized	16	4.0 ± 0.7	2.4 ± 0.4	1.6 ± 0.4	1.3 ± 0.4	− 68	1.4 ± 0.7
5. Reserpine (100 μg), ovariectomized	13	4.3 ± 0.6	3.6 ± 0.5	1.4 ± 0.3	–	–	–

[aa] DMBA was administered at 55 days of age. The rats were treated with reserpine and/or ovariectomized approximately 75 days after DMBA treatment. [bb] S.E. of the mean. [a,c], [d,f], [g,i], [j,k] = P 0.001. [a,b] = P 0.02. [d,e], [g,h] = P 0.05.

existing rat MT[7,8]. Reserpine, however, failed to significantly influence MT development in ovariectomized rats. This may be due, at least in part, to inadequate prolactin secretion by these rats. Recent studies in our laboratories have demonstrated that reserpine may or may not have a significant effect on blood prolactin levels in ovariectomized rats[9], depending upon the dose and time schedule of administration.

The lower dose of reserpine administered either before or after MT appearance had no effect on body weight. The higher dose of reserpine given before MT appearance decreased body weight for a period after treatment but most rats recovered by the time the experiments were terminated. The temporary loss in body weight may have been partly responsible for the decreased incidence of MT observed in these rats, since losses in body weight are often associated with decreased tumor development[10]. The higher dose of reserpine administered to ovariectomized MT-bearing rats for 25 days markedly reduced body weights, and as a result this treatment was not continued for 50 days or administered to intact-MT bearing rats.

LACASSAGNE and DUPLAN[11] reported that mice of the C_3H strain, treated with reserpine, developed spontaneous MT earlier than the controls. These results are in accord with the study of BOOT et al.[12] which demonstrated that long term injections of prolactin in mice resulted in enhanced mammary tumorigenesis. By contrast, CRANSTON[13] reported that several central nervous system depressants, including reserpine, had no significant effect on growth of spontaneous MT in mice. DUKOR et al.[14] reported that the maximum tolerated dose of reserpine given to mice bearing spontaneous MT had a slight inhibitory effect which could be correlated with the degree of body weight loss. The probable explanation for these contrasting results is that the mouse MT are not generally hormone responsive in later stages of development, although they are initially hormone dependent[15]. On the other hand, carcinogen-induced rat MT are hormone dependent initially and generally remain hormone responsive in later stages of development, since either hypophysectomy or ovariectomy before or after MT appearance results in decreased incidence and growth, respectively, of MT[16].

Résumé. Si l'on injecte de la réserpine à des rates avant de les avoir traitées au carcinogène, leurs tumeurs mammaires diminuent très nettement. En revanche, si l'injection de réserpine s'opère chez des rates déjà atteintes de ces tumeurs, la croissance de ces dernières en est sensiblement stimulée.

C. W. WELSCH and J. MEITES

Departments of Anatomy and Physiology, Michigan State University, East Lansing (Michigan 48823, USA), 16 March 1970.

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Extranuclear ^{3}H-Thymidine Incorporation in Subcortical Cytoplasmic Organelles of Primary Oocytes in the Japanese Quail (*Coturnix coturnix japonica*)

In the present work, laying Japanese quails were injected i.p. with 1 mCi of thymidine 6-^{3}H (specific radioactivity: 14 Ci/m*M*, 1 mCi/ml). $1^1/_2$ h later, the animals were killed by decapitation, their abdomens opened and the intrafollicular peduncular oocytes removed from the ovaries by the cutting off their pedicle. These oocytes were then fixed in acetic acid-alcohol (1:3) for 1 h at 4 °C, thereafter for 2 h at room temperature. After dehydration, the oocytes were embedded in paraffin and sectioned at 8 μ. After deparaffination and rehydration, the acid-soluble precursors were extracted by treatment of the sections with 3% perchloric acid at 4 °C for 20 min. The slides were coated with nuclear emulsion L4 (Ilford, Great Britain) by the dipping method. After 28 days