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MAMMARY TUMORS AND SERUM HORMONES IN THE BITCH TREATED WITH MEDROXYPROGESTERONE ACETATE OR PROGESTERONE FOR FOUR YEARS

DOYLE W. FRANK, D.V.M., PH.D.*
KENNETH T. KIRTON, PH.D.†‡
THOMAS E. MURCHISON, D.V.M., PH.D.§
WILLIAM J. QUINLAN, PH.D.¶
MARTIN E. COLEMAN, PH.D.§
TERRY J. GILBERTSON, PH.D.†
ERNEST S. FEENSTRA, D.V.M., PH.D.†
FRANCES A. KIMBALL, PH.D.†

The Upjohn Company, Kalamazoo, Michigan 49001, Dawson Research Corporation, Orlando, Florida 32806, and Department of Radiation Biology and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, New York 14642

After 4 years of a long-term contraceptive steroid safety study, the incidence and the histologic types of mammary dysplasia produced are shown to be similar in beagles treated with medroxyprogesterone acetate (medroxyprogesterone) or progesterone.

Serum insulin, thyroid-stimulating hormone (TSH), triiodothyronine, growth hormone, prolactin, 17β-estradiol, progesterone, and cortisol were determined by radioimmunoassay on samples collected after 45 months of treatment. Serum growth hormone and insulin concentrations were elevated in a dose-related manner in both treatment groups. Levels of triiodothyronine, cortisol, and 17β-estradiol (medroxyprogesterone only) were lowered. TSH and prolactin concentrations were not changed. Pituitary-gonadal hormone interaction in the pathogenesis of mammary neoplasia of the dog is discussed. Prolonged treatment of beagles with doses of progesterone or medroxyprogesterone 1 to 25 times the human contraceptive dose or luteal phase (dog) levels, respectively, results in a dose-related incidence of mammary nodules. Fertil Steril 31:340, 1979

The hormone dependence of mammary cancer was hypothesized in 1896 by Beatson,[1] who noted remission of tumors in patients following ovariectomy. The experimental induction of mammary tumors in mice bearing pituitary isografts[2] suggested a relationship between steroid and protein hormones in tumor development and permitted investigation of the prolactin-estrogen interaction in the laboratory study of mammary neoplasia. The pathogenesis of mammary neoplasia has been intensively examined in rodents, and is only partly understood in women.[3, 4] However, in the dog, a species with a high spontaneous incidence of mammary dysplasia[5, 6] and neoplasia,[7, 8] it remains incompletely characterized.

Included among the tests required for safety assessment of steroidal contraceptives are 7-year studies in the beagle, at fixed multiples of the

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*The Upjohn Company, Kalamazoo, Mich. Present address: Dawson Research Corporation, P.O. Box 8272, Orlando, Fla. 32856.

†The Upjohn Company, Kalamazoo, Mich. 49001.

‡To whom reprint requests should be addressed at the Upjohn Company.

§Dawson Research Corporation, P.O. Box 8272, Orlando, Fla. 32806.

¶Department of Radiation Biology and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y. 14642.

proposed human dose.[9] A tumorigenic response was observed within the first 4 years of this study. This evidence that mammary cancer in other species may be hormone-dependent prompted us to assay hormones in serum samples of all animals and to classify mammary dysplasia and neoplasia in dogs which have died to date. This report included the results of the first 4 years, the approximate midpoint of the study.

The 17α-hydroxyprogesterone derivatives used alone have produced no increase in the incidence of mammary tumor in rats,[10] mice,[10] or monkeys,[11] and no evidence of mammary dysplasia has been found in women given medroxyprogesterone acetate when used alone in clinical contraceptive testing.[12] Contraceptive doses of medroxyprogesterone have not been linked to alterations of prolactin[13] or growth hormone secretion[14] or to abnormally elevated serum 17β-estradiol[15] in clinical trials of the drug. However, an increased incidence of mammary dysplasia has been found in the dog.

MATERIALS AND METHODS

Animals and Drug Administration. One hundred and forty virgin beagle bitches were hysterectomized at 4 to 6 months of age and separated into six treatment groups. Medroxyprogesterone acetate (medroxyprogesterone) was administered intramuscularly as an aqueous suspension. Drug-treated groups of 20 bitches each received 30, 180, or 690 mg of medroxyprogesterone/dog every 3 months for 48 months. The doses correspond to 1×, 6× and 23× the human contraceptive dose, as required by Food and Drug Administraton (FDA) guidelines. Approximately 50 mg/dog is the efficacious contraceptive dose in the bitch.[16] Two other groups of 20 dogs each received intramuscularly an aqueous suspension of 46 or 1140 mg of progesterone/dog/week. Preliminary evaluation suggested that these progesterone doses resulted in approximately 20 or 500 ng/ml of serum progesterone, which corresponds to approximately 1× or 25× luteal phase concentrations in the dog. Forty dogs received aqueous vehicle every 3 months through the 29th month of study. After 29 months, the control animals were separated equally, with one-half receiving the vehicle volume equivalent to the high-dose progesterone group (i.e., 11.4 ml/dog/week) and the other half receiving vehicle volume equivalent to that administered to the high-dose medroxyprogesterone group (i.e., 4.6 ml/dog/3 months).

The animals were housed individually in screened outdoor runs and allowed water ad libitum. The standard laboratory chow ration was adjusted to maintain a desirable body weight. The average group weight was 8.4 ± 0.3 kg at the beginning of study and 8.9 ± 1.2 kg after 4 years.

Routine Observations. Clinical observations were conducted daily, and estrous and general health examinations were conducted weekly. The mammary glands were examined monthly by visual inspection and palpation for changes in numbers and character of mammary nodules and the character of discharge. Since inception of the study, the same person has conducted these examinations and updated schematics and descriptions of the nodules. Mammary nodules which were less than 2 mm in diameter and, therefore, no longer palpable, were deleted from the record the following month. As the individual nodules became confluent, thus losing their individual identity, they were scored as originally found. Tissue for examination was derived from unscheduled deaths; the nodules were not biopsied since FDA guidelines do not permit tissue biopsy during a long-term toxicology study. (The mammary nodules were histologically typed according to the classification proposed by Hampe and Misdorp[17] and in accordance with the guidelines developed by the Armed Forces Institute of Pathology (AFIP), Contraceptive Steroid Project. Contributors to the Contraceptive Steroid Project include industrial pathologists and staff of the AFIP and FDA. A project goal was the development of diagnostic criteria for mammary tumors of the bitch.) Routine hematology, serum chemistry, ophthalmology, bromsulfophthalein retention, and oral glucose tolerance tests were performed at regular intervals.

Hormone Determinations. Serum samples were collected and frozen during the 45th study month from all surviving dogs. All hormones were quantitated by radioimmunoassay. Canine prolactin was determined by a heterologous assay[18] employing an ovine prolactin standard. Canine growth hormone was determined by an assay employing rhesus monkey antibody to canine pituitary growth hormone.[19] Thyroid-stimulating hormone (TSH) was determined by radioimmunoassay described by Quinlan and Michaelson.[20] Progesterone,[21] cortisol,[22] triiodothyronine (T-3),[23] and 17β-estradiol[24] were determined by previously published procedures. Insulin was quantitated with a Schwarz/Mann kit (Orangeburg, N. J.).

Statistical Analysis. Analysis of variance was used to test for over-all differences among treat-

TABLE 1. *Incidence of Mammary Nodules in Beagles Treated with Medroxyprogesterone Acetate and Progesterone for Four Years*

Dose group	No. of surviving dogs[a]	No. of dogs with nodules	No. of nodules present	No. of nodules no longer present[b]
Vehicle controls, medroxyprogesterone acetate	17	2	2	1
Vehicle controls, progesterone	18	0	0	1
Medroxyprogesterone acetate, 3 mg/kg	19	13	29	6
Medroxyprogesterone acetate, 30 mg/kg	18	15	93	15
Medroxyprogesterone acetate, 75 mg/kg	14	12	105	26
Progesterone, 5 mg/kg	20	13	26	14
Progesterone, 125 mg/kg	4	4	45	10

[a]Initially 20 dogs per group.
[b]Nodules no longer clinically detected.

ment group responses. Where applicable for statistically significant end-points, the response of each treatment group was compared with that of the control. When treatment group variances were not homogeneous, a log transformation of the data was utilized. If variance remained heterogeneous, the Kruskal-Wallis test was used to test for over-all differences among treatment group responses. For statistically significant end-points in this case, each treatment group was compared with the control using the Wilcoxon rank sum test.[25] It should be noted that when the Wilcoxon test was used, the probabilities presented refer to pairwise comparisons and do not account for experimental error rates except when the results of the medroxyprogesterone groups pooled were compared with the results of the progesterone groups pooled.

RESULTS

The results of the 48th-month mammary gland clinical examinations are summarized in Table 1, and the histologic diagnosis of nodules from dogs which have died is found in Table 2. The incidence (Table 1) and type (Table 2) of tumors produced by medroxyprogesterone and progesterone and the number of nodules per animal were similar. The distribution of tumor type within treatment group was not uniform, varying from zero to the total number for the group. Peripheral serum hormone concentrations in samples collected during the 45th month from dogs treated with medroxyprogesterone or progesterone are illustrated in Tables 3 and 4, respectively. Serum prolactin, growth hormone, and insulin responses were similar in the two treatment groups. Prolactin concentration did not change with treatment, whereas growth hormone and insulin concentrations increased in a dose-related manner in both progestin-treated groups. Triiodothyronine was depressed in dogs treated with either progestogen, and thyroid-stimulating hormone was significantly depressed in the high-dose medroxyprogesterone-treated group. Serum progesterone was increased in the progesterone-treated groups, as expected, and in the low-dose medroxyprogesterone-treated groups as well. Serum estradiol levels were similar in all progesterone-treated groups, but were depressed in a dose-related manner in medroxyprogesterone-treated dogs. Serum cortisol was decreased significantly in dogs treated with progesterone and in the high-dose medroxyprogesterone-treated group. There were no significant differences in the hormone values of vehicle control dogs for progesterone versus vehicle control dogs for medroxyprogesterone with the exception of significantly increased serum cortisol levels in the progesterone control group ($P < 0.05$). Pooled data for the progesterone-treated groups, as compared with the pooled results of medroxyprogesterone-treated groups, revealed significantly increased estradiol and progesterone levels in dogs treated with progesterone ($P < 0.01$). Other responses were similar in groups of dogs treated with either progestogen.

DISCUSSION

The incidence of mammary nodules in bitches treated with progesterone and medroxyprogesterone for 4 years was similar and related to the dose of the compound administered. Microscopic examination of the mammary nodules indicated a similar histologic spectrum and distribution of change in both progesterone- and medroxyprogesterone-treated bitches. Lobular hyperplasia was

TABLE 2. *Histopathology of Mammary Nodules in Beagles Treated with Medroxyprogesterone Acetate and Progesterone for Four Years*[a]

Types of lesions	Vehicle for progesterone	Medroxyprogesterone acetate (mg/kg)			Progesterone (mg/kg)	Total
		3	30	75	125	
No. of dogs examined	2	1	2	2	16	23
Hyperplasias						
Simple lobular hyperplasia	2	1	3	9	11	26
Complex lobular hyperplasia	0	0	3	13	33	49
Simple papillary cyst	0	0	5	4	4	13
Complex papillary cyst	0	0	2	0	0	2
Total hyperplasias	2	1	13	26	48	90
Tumors						
Simple adenoma	0	0	1	1	1	3
Complex adenoma	0	0	4	12	9	25
Mixed mammary tumor	0	0	0	4	1	5
Complex duct papilloma	0	0	0	1	1	2
Simple duct papilloma	0	0	0	1	0	1
Basiloid adenoma	0	0	0	0	1	1
Intralobular noninfiltrating solid carcinoma, comedo type[b]	0	0	0	0	1	1
Focus of noninfiltrating carcinoma	0	0	0	0	1	1
Total tumors	0	0	5	19	15	39
Total nodules	2	1	18	45	63	129

[a]Nodules were classified according to the scheme of Hampe and Misdorp[17] as modified by the Contraceptive Steroid Project at the Armed Forces Institute of Pathology. All deaths were unscheduled; see Table 1 for number of dogs surviving at 4 years. None of the three animals in the medroxyprogesterone acetate control group had mammary nodules, and no deaths occurred in the group receiving 5 mg/kg of progesterone.

[b]Not a nodule.

the most frequently observed microscopic lesion, and complex adenomas were the neoplasms found most frequently. No morphologic evidence was found that tumors associated with medroxyprogesterone treatment differed from those produced by progesterone except that histologic evidence of

TABLE 3. *Serum Hormone Determinations in Beagles Given Medroxyprogesterone Acetate for 45 Months*

Hormone	Vehicle control	Medroxyprogesterone acetate			Over-all significance
		3 mg/kg	30 mg/kg	75 mg/kg	
Insulin (μU/ml)	5.98 ± 4.55[a] (15)	15.60 ± 5.60[b, c] (18)	23.42 ± 17.14[b, c] (20)	28.39 ± 18.19[b, c] (16)	b, c
TSH (ng/ml)	6.15 ± 3.23 (17)	5.60 ± 3.92 (20)	6.05 ± 3.57 (20)	3.01 ± 1.68[b, c] (16)	b, c
Thyroxin (T_3) (ng/100 ml)	130.52 ± 25.79 (17)	116.25 ± 41.59 (20)	104.09 ± 47.01[d, e] (20)	104.75 ± 22.29[b, e] (16)	d, f
Growth hormone (ng/ml)	5.67 ± 1.27 (14)	6.77 ± 5.23[d, e] (12)	42.45 ± 48.84[b, e] (16)	52.14 ± 47.49[b, e] (12)	b, f
Prolactin (ng/ml)	1.60 ± 0.63 (15)	2.20 ± 0.95 (20)	1.70 ± 0.65 (20)	2.06 ± 1.48 (16)	
17β-Estradiol (pg/ml)	29.12 ± 7.29 (16)	22.65 ± 7.00[d] (20)	18.22 ± 6.86[b] (20)	12.68 ± 4.77[b] (16)	b
Progesterone (ng/ml)	9.9 ± 15.2 (17)	34.3 ± 70.9[b, c] (20)	3.3 ± 4.5 (19)	4.9 ± 4.6 (16)	b, c
Cortisol (μg/100 ml)	2.27 ± 1.04 (17)	2.08 ± 1.18 (20)	2.89 ± 2.12 (20)	0.57 ± 0.42[b, f] (16)	b, f

[a]Means ± standard deviation; numbers of observations are shown in parentheses.

[b]$P < 0.01$.

[c]Significance determined by log transformation of data.

[d]$P < 0.05$.

[e]Significance determined by Wilcoxon's rank sum test.

[f]Significance determined by Kruskal-Wallis test.

TABLE 4. *Serum Hormone Determinations in Beagles Given Progesterone for 45 Months*

Hormone	Vehicle control	Progesterone		Over-all significance
		5 mg/kg	125 mg/kg	
Insulin (μU/ml)	6.63 ± 5.71[a] (15)	13.51 ± 9.14[b, c] (20)	27.24 ± 15.46[c, d] (7)	c, d
TSH (ng/ml)	5.67 ± 2.22 (17)	6.63 ± 6.16 (20)	4.40 ± 3.38 (7)	
Thyroxin (T_3) (ng/100 ml)	125.88 ± 38.13 (18)	115.39 ± 39.03 (20)	74.00 ± 35.06[d, e] (7)	b
Growth hormone (ng/ml)	6.92 ± 1.89 (11)	5.37 ± 2.09[b, e] (20)	89.20 ± 88.96[d, e] (5)	d, f
Prolactin (ng/ml)	1.70 ± 0.68 (17)	1.80 ± 0.61 (20)	1.85 ± 0.37 (7)	
17β-Estradiol (pg/ml)	26.20 ± 9.35 (18)	24.55 ± 7.72 (20)	29.69 ± 13.09 (7)	
Progesterone (ng/ml)	13.8 ± 25.2 (18)	62.06 ± 159.7[d, e] (20)	156.3 ± 67.1[d,e] (7)	c, d
Cortisol (μg/100 ml)	3.11 ± 0.87 (18)	2.02 ± 0.98[d] (20)	1.45 ± 1.21[d] (6)	d

[a]Means ± standard deviaton.
[b]$P < 0.05$.
[c]Significance determined by log transformation of data.
[d]$P < 0.01$.
[e]Significance determined by Wilcoxon's rank sum test.
[f]Significance determined by Kruskal-Wallis test.

malignancy was confined to two dogs treated with 125 mg/kg of progesterone weekly for 4 years. These findings must be interpreted with caution, since the histologic data were obtained only from unscheduled deaths. They do represent an appropriate midstudy analysis of potentially important mammary changes induced by progestin therapy in the bitch.

The biologic significance of progestogen stimulation to mammary tumorigenesis of the bitch is indicated by the strong dose relationship between the treatment and nodule incidence (Table 1). Prolonged progesterone treatment with dose levels 25× normal luteal phase levels is tumorigenic in the beagle. Similarly, medroxyprogesterone acetate (17α-hydroxy-6α-methylprogesterone acetate) is tumorigenic in the beagle when administered at multiples of the human dose prescribed for steroid contraceptive safety assessment (Table 1). This is in keeping with the previously reported responsiveness of the canine mammary gland to stimulation by progestational agents.[26]

In their recent review, McGuire et al.[27] indicated that the relationship of progesterone with anterior pituitary hormones in the genesis of mammary neoplasia is unclear. Evidence from studies of rats and mice suggests a primary relationship between estrogen and prolactin in the development of mammary neoplasia, whereas progesterone plays an important but uncertain role. Although approximately 40% of breast cancer in women is responsive to steroid chemotherapy, the role of pituitary hormones in the genesis of mammary neoplasia in women is based largely upon circumstantial evidence. Higher serum prolactin levels have been reported in families with an increased incidence of breast cancer; however, prolactin receptors have not been demonstrated in human breast cancer tissue.[27] Hormonally responsive tumors are thought to be primarily estrogen-dependent. Estrogen, however, increases progesterone receptors in mammary tissue, and the clinical remission rate reflects an advantage of combined steroid chemotherapy upon the demonstration of estrogen and progesterone receptors. Contraceptive doses of medroxyprogesterone in women do not produce alterations of prolactin[13] or growth hormone secretion,[14] or result in abnormal levels of serum 17β-estradiol.[15]

In the present study, serum prolactin levels were similar in all groups of dogs, which suggests that chronic progestational stimulation does not result in increased prolactin secretion in the bitch. Growth hormone was increased by both progestogens in a dose-related fashion. Because many dogs receiving high doses showed alterations in carbohydrate metabolism, elevated serum insulin levels, and acromegalic changes, this increase in growth hormone was not unexpected. Serum prolactin shows no apparent relationship to the incidence of progestationally induced mammary nodules of the bitch, whereas serum growth hormone shows a close relationship to the incidence of mammary nodules. Gräf[28] reported similar

findings in beagles given norethisterone oenanthate. (In spontaneous mammary carcinoma of the dog, Hamilton et al.[29] found no difference in serum prolactin levels in 32 dogs with neoplasia and 22 normal dogs.) From direct analysis of the dog pituitary in various physiologic and pathologic states, Saluja et al.[30] concluded that estrogen is prolactin-responsive in the dog as well as in the rat. Increased prolactin concentrations occurred in the pituitaries of 9 of 12 bitches with aggressive mammary carcinoma; however, peripheral prolactin concentrations were not determined. The pituitary growth hormone content also was not reported.

Our observations and those of others[31] indicate that mammary tumors in the bitch may be expressed as a result of prolonged stimulation with large doses of progesterone or closely related 17α-hydroxysteroid derivatives. Similar results with these compounds have not been reported in other species. No evidence of treatment-related mammary dysplasia has been seen in clinical trials with medroxyprogesterone acetate.[12]

The results of these studies reveal several significant differences (and some similarities) between beagles and humans in the hormonal response to exogenous progestational compounds. In the present study, serum prolactin and cortisol levels were not changed significantly in dogs receiving 3 mg/kg of medroxyprogesterone, in accord with previous reports for the human.[13, 15] Growth hormone was elevated and estradiol depressed ($P < 0.05$) in the dog, whereas these parameters were not changed in the human.[14, 15] Progesterone was elevated in the low-dose medroxyprogesterone-treated dogs, which may reflect continued ovarian cyclicity. This is in contrast to human studies.[15] Basal serum insulin was elevated in the dog ($P < 0.01$), but was elevated only following a glucose tolerance test in the human.[14]

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