Estrogen Receptors in Canine Mammary Tumors

E. G. MacEwen, A. K. Patnaik, H. J. Harvey, and W. B. Panko

ABSTRACT

The presence of estrogen receptor in 67 canine mammary lesions was correlated with pathological features of the disease. All tissue specimens were analyzed for estrogen receptor content by a sucrose gradient ultracentrifugation method previously used in analyzing human breast cancer cytosols. Pathological features of the tissues were assessed by a veterinary pathologist without knowledge of results of estrogen receptor analysis. Sixty-two (92.5%) of the tissue samples analyzed were classified as epithelial neoplastic lesions, and 38 of these (61.3%), including 24 adenocarcinomas, were estrogen receptor positive (i.e., estrogen receptor concentration equal to or greater than 10 fmol/mg cytosol protein). All five of the non-epithelial neoplastic lesions were estrogen receptor negative.

Canine and human breast cancers share common histological types and have similar biological behavior. If a significant percentage of canine mammary cancer is also estrogen dependent, the dog may be a useful model for hormonal studies and for the development of models of endocrine therapy for human breast cancer.

INTRODUCTION

That endocrine therapy affects the growth of human breast cancer is well known (5). Furthermore, even breast tumors of a single histological type frequently manifest a wide spectrum of biological behavior. Breast cancers can be classified as ER-positive or negative (3) or "poor" on the basis of biochemical measurements of the concentrations of ER (12). These 2 classes have distinct biological characteristics; the most important is that about 50% or more of ER-rich tumors respond to endocrine therapy, while ER-poor tumors infrequently respond to such therapy (5, 6, 15).

Animal models of hormone-dependent breast cancer have been restricted to either spontaneous or induced mammary tumors occurring in rodents (1, 2). Canine mammary cancer is a spontaneously occurring disease believed to be influenced by hormones (9). Reduction of the risk of breast cancer in aging individuals as the result of ovariectomy relatively early in life is an epidemiological factor characteristic of human breast cancer (8) and of canine disease as well (22). Additional epidemiological factors in canine mammary cancer similar to those reported in humans are the age-related incidence, the protective effect of early first pregnancy or pseudopregnancy, and eosin.

Rodent models of human breast cancer have proved useful in the development of models of endocrine therapy and have also yielded much insight into the basic biological mechanism of hormone action in human breast cancer (2). Presently available data suggest that the combination of cytotoxic chemotherapy and various endocrine manipulations may be the optimal mode of therapy for advanced breast cancer (6). However, the rodent models are not entirely applicable to human breast cancer, particularly the carcinogen-induced tumors. Additional animal models of human breast cancer may be useful in developing and optimizing this combination of therapy. If canine mammary cancer, particularly adenocarcinoma, is truly an endocrine-dependent disease, the dog may become a useful model for treatment combinations that include hormonal therapy (23).

The purpose of this study was to correlate the presence of ER in a series of canine mammary lesions with pathological features of the disease. The clinical follow-up of the dogs is continuing and subsequently will be reported in more detail.

MATERIALS AND METHODS

From January 1978 to January 1979, 67 privately owned dogs with spontaneous mammary lesions were seen at the Animal Medical Center for breast masses. All dogs underwent a complete physical examination, hemogram, serum chemistry profile (SMA 12/60 Auto-Analyzer, Technicon Instruments, Tarrytown, N. Y.), and chest radiography for evidence of metastatic disease. All dogs were clinically staged as: Stage I, primary tumor without regional lymph node metastasis; Stage II, primary tumor with histological evidence of lymph node metastasis; or Stage III, primary tumor with distant metastasis.

All breast lesions were removed by mastectomy. Tumors were measured, and adipose and necrotic tissue was removed. Samples were taken for the measurement of ER content and stored at —70° within 15 min after surgical removal. The remaining tissue was fixed in 10% buffered formalin, processed routinely, and stained with hematoxylin and eosin.

Pathological features of the tissues were assessed by a pathologist (A. K. Patnaik) without knowledge of results of ER analysis. The tumors were histologically classified according to the WHO classification scheme for canine mammary cancer (11). Lesions were classified as inflammatory (mastitis), benign (adenomas, with or without carcinoma in situ), or malignant (adenocarcinoma or sarcoma). The adenocarcinomas were further subdivided into tubular, mucinous, spindle cell, solid, or papillary adenocarcinoma.

ER Assay: ERs in canine mammary cancer specimens were quantitated by a prelabeled sucrose gradient method previously designed for use in human breast cancer (19). Briefly, the tumor specimen was shattered while being maintained at a temperature of less than —60° with a Thermovac tissue pulverizer (Glass Seal Division, Thermovac Industries, Inc., Copiague, N. Y.). The resulting fine powder was suspended in 5 volumes of 10 mM monothioglycerol:0.01 M Tris-HCl buffer, pH 7.4, 4°. All remaining steps were carried out at 4° unless otherwise indicated. The suspension was homogenized 3 times for 10

1 This work was supported by Grants CA-19072, CA-25586, and CA-26452 from The National Cancer Institute, the Cancer Research Institute, and The Bodman Foundation. A preliminary report of these data was presented at the First International Congress on Hormones and Cancer, October 3 to 6, 1979, at Rome, Italy [S. Iacobellis, R. J. B. King, H. R. Lindner, and M. E. Lippman (eds.), Hormones and Cancer. New York: Raven Press, 1980].

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3 The abbreviations used are: ER, estrogen receptor; DES, diethylstilbestrol.

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sec with a Tissuezizer TDT-10 (Tekmar Industries, Cincinnati, Ohio) at a setting of 35. Pauses of 30 sec were observed between homogenization steps.

The homogenate was centrifuged for 10 min at 800 × g, and the supernatant was reserved. The supernatant was centrifuged for 30 min at 105,000 × g in a type 50 rotor in a Beckman L5-50 ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.). Aliquots were set aside for protein determination by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.), a modification of the method of Bradford (3), using recrystallized bovine serum albumin for a standard.

Cytoplasmic ERs were determined in the following manner. Aliquots (0.15 ml) of the fractions were incubated for 4 hr in a total volume of 1 ml. The intraassay variation ranges from 8 to 10% and the interassay variation ranges from 9 to 14%.

In addition, some of the tumor cytosols (singly or pooled) were subjected to saturation analysis: cytosol from several malignant tumors was incubated with varying concentrations of 17β-[3H]estradiol with and without unlabeled ligand. The binding of 17β-[3H]estradiol to the cytosol receptor was determined by the dextran-coated charcoal method (19), and the resulting binding data were replotted by the method of Scatchard (21).

A tumor was considered positive for ER if the ER concentration exceeded 10 fmol/mg cytosol protein. The positive range was further subdivided into 1+ (10 to 19 fmol/mg), 2+ (20 to 49 fmol/mg), 3+ (50 to 99 fmol/mg), or 4+ (100 or more fmol/mg). Tumors were considered negative for ER if the concentration was less than 10 fmol/mg.

Tumor volume was also correlated with ER analysis. Tumor volume in cu cm was determined after removal by obtaining 3-dimensional measurements with calipers.

RESULTS

Analysis of Putative ER. Cytosol was analyzed for the presence of putative ER content. Results are shown in Chart 1. For saturation analysis, cytosol from a tumor suspected of containing ER was incubated with 17β-[3H]estradiol, with and without the presence of a 100-fold excess of 17β-estradiol, DES, progesterone, cortisol, or dihydrotestosterone. It can be seen in Chart 1A that only the 2 estrogens inhibited the binding of the radioactive ligand to a macromolecular component of the cytosol. Using individual tumors, peaks of 17β-[3H]estradiol binding corresponding to macromolecular components sedimenting predominantly at 8S (Chart 1B) or 4S (Chart 1C), are also shown. Chart 1B also shows that the inclusion of 0.4 M

![Image of chart 1](chart1.png)
KCI in the gradient results in the conversion of the 8S species to a component sedimenting at approximately 4S. Chart 1D shows the effect of Pronase or heat treatments on the specific binding of 17β-[3H]estradiol to macromolecular component.

The results of saturation analysis of canine mammary cancer cytosol are shown in Chart 2A, and replotting in Scatchard coordinates is shown in Chart 2B. From this Scatchard plot (21), the apparent dissociation constant (Kd) was determined to be 0.67 nM, and the concentration of binding sites was 0.23 pmol/ml (64 fmol/mg cytosol protein). The Kd was determined for 5 canine tumors, and the mean ± S.E. was calculated to be 0.59 ± 0.17 nM.

No significant 17β-[3H]estradiol binding was detected in cytosol derived from canine muscle, lung, or diaphragm. No detectable 17β-[3H]estradiol binding was found in cytosol from inflammatory canine mammary lesions or nonepithelial neoplastic lesions (sarcoma).

Analysis of Canine Mammary Lesions. Sixty-six of the 67 dogs evaluated were female (2 were spayed), and one was an intact male. The median age of the dogs was 11 years, with a range of 5 to 15 years. With respect to breed, the most commonly represented were poodle, terrier, and mixed.

Sixty-seven dogs were clinically staged: 65 dogs were Stage I (local disease); and the other 2 dogs were Stage II (regional metastasis). A histological classification of tumors and ER content (positive or negative) is given in Table 1. Of the 67 samples analyzed, 62 (92.5%) were classified as epithelial neoplastic lesions, i.e., adenocarcinoma, adenoma, or carcinoma in situ. Thirty-eight of these (58.4%), including 24 adenocarcinomas, were ER positive. All 5 of the nonepithelial neoplastic lesions were ER negative. A striking feature (Table 1) is that, while 52.3% (24 of 46) of the adenocarcinomas were ER positive, 87% (7 of 8) of both the adenomas and carcinomas in situ were ER positive. Table 2 contains the mean ER values and ER ranges for the different categories of epithelial neoplastic lesions. The distribution of the different amounts of ER content (+1, +2, etc.) according to histological type is given in Table 3.

The histological classification of the adenocarcinomas along with ER status is given in Table 4. Tubular adenocarcinoma was the largest group, followed by solid adenocarcinoma. Because of the limited number of cases, statistically significant results were not obtainable, but one interesting trend appeared. Solid adenocarcinomas were ER positive 30% of the time (3 of 10), while the other histological types were ER positive 50 to 83% of the time.

Preliminary follow-up data from the 67 cases reported here have been obtained and are given in Table 5. Forty-three dogs are alive with no evidence of disease; 5 dogs (7.4%) are alive with recurrent metastasis; and 7 dogs (10.4%) have died. Three of the 7 dogs that died of cancer were ER negative (42.8%). Inasmuch as the median duration of follow-up is only 7 months and the number of cases studied is relatively small, conclusions about the correlation of ER status and disease-

<table>
<thead>
<tr>
<th>Histological type</th>
<th>ER positive</th>
<th>ER negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>24 (52.3%)</td>
<td>22 (47)</td>
<td>46 (68.6)</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>7 (13)</td>
<td>1 (2)</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>0</td>
<td>3 (100)</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Mastitis</td>
<td>0</td>
<td>2 (100)</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (58.4)</td>
<td>29 (41.6)</td>
<td>67 (100)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. of ER positive*</th>
<th>Mean ± S.E.</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
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<tr>
<td>Adenocarcinoma</td>
<td>24</td>
<td>21.6 ± 11.1</td>
<td>10</td>
<td>60.3</td>
</tr>
<tr>
<td>Adenoma</td>
<td>7</td>
<td>22.4 ± 8.5</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>7</td>
<td>31.3 ± 16.4</td>
<td>10.5</td>
<td>109</td>
</tr>
</tbody>
</table>

* 10 fmol/mg cytosol protein or greater.
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Table 3

<table>
<thead>
<tr>
<th>ER content (fmol/mg)</th>
<th>No. of lesions</th>
<th>Adenocarcinoma</th>
<th>Adenoma</th>
<th>Carcinoma in situ</th>
<th>Sarcoma</th>
<th>Other</th>
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<tbody>
<tr>
<td>0-9.9</td>
<td>29</td>
<td>22</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>10-19.9</td>
<td>12</td>
<td>7</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
<td>20-29.9</td>
<td>20</td>
<td>14</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30-49.9</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>50+</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>46</td>
<td>8</td>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Histological type</th>
<th>ER positivea</th>
<th>ER negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular</td>
<td>11 (50)%</td>
<td>11 (50)</td>
<td>22 (44.5)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>5 (83)</td>
<td>1 (17)</td>
<td>6 (13.6)</td>
</tr>
<tr>
<td>Spindle cell</td>
<td>2 (66.6)</td>
<td>1 (33.3)</td>
<td>3 (6.6)</td>
</tr>
<tr>
<td>Solid</td>
<td>3 (30)</td>
<td>7 (70)</td>
<td>10 (22.7)</td>
</tr>
<tr>
<td>Papillary</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td>5 (11.3)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (53.5)</td>
<td>22 (46.7)</td>
<td>46 (100)</td>
</tr>
</tbody>
</table>

Table 5

Preliminary follow-up data on 67 dogs with mammary lesionsa

<table>
<thead>
<tr>
<th>ER concentration (fmol/mg protein)</th>
<th>No. of dogs</th>
<th>Aliveb</th>
<th>With disease</th>
<th>From disease</th>
<th>Other causes</th>
<th>Lost to follow-up</th>
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</thead>
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<tr>
<td>50 or more</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20-49.9</td>
<td>20</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>10-19.9</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>9.9 or less</td>
<td>29</td>
<td>22</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>43 (64)c</td>
<td>5 (7.4)</td>
<td>7 (10.4)</td>
<td>4 (6.3)</td>
<td>8 (11.9)</td>
</tr>
</tbody>
</table>

a fmol/mg cytosol protein or greater.
b Numbers in parentheses, percentage.
c Median follow-up time of 7 months.

DISCUSSION

This report documents the presence of ER in epithelial neoplastic lesions of the canine mammary gland by demonstrating that the 17β-[3H]estradiol binding found in canine mammary tissue cytosol meets the criteria for receptors, i.e., high-affinity, low-capacity ligand binding with appropriate steroid and tissue specificity. There have been previous reports of specific receptors for 17β-[3H]estradiol in canine mammary cancer, although one of these provided insufficient data to support the contention (7, 10, 18). One of these reports also recorded the presence of androgen and progesterone receptors (10).

Thirty-eight of 62 epithelial neoplastic lesions of the canine mammary gland were ER positive; 24 of 46 adenocarcinomas (52%) found among these neoplastic lesions were ER positive. Compared with a series of over 1000 malignant breast tumors from women analyzed over the past 4 years in the laboratory of W. B. Panko by the same method used in this study, there was no significant difference in the proportion of ER-positive breast cancers in the 2 different populations.

The analysis of ER distribution shows that the canine mammary tumors tend to have fewer ER values in the high end of the range (100 fmol/mg or more); however, the results do not quite reach statistical significance (p = 0.17). Benign tumors, representing 24.6% of the epithelial neoplastic lesions, were ER positive 87% of the time (14 of 16). This is in agreement with previously reported studies in humans. Benign tumors (usually fibroadenomas) from women are ER positive only 10 to 20% of the time (20). However, in one study of breast lesions, approximately 50% of the benign tumors were ER positive (14). It is interesting to speculate that benign lesions may have a higher level of ER because they are better differentiated. There is ample support in the literature for this hypothesis (16). Why this is not consistently observed in benign lesions of the human breast but is apparently true for benign canine mammary lesions is a question worthy of further study.

All 3 sarcomas investigated in this study were ER negative. This is to be expected because of the lack of epithelial elements in these lesions.

Tubular adenocarcinomas were the most common histological subtype, followed by solid adenocarcinomas. Solid adenocarcinomas tend to be aggressive, and 70% (7 of 10) in this study were ER negative. This compares to ER-negative human breast cancer which is also aggressive, as evidenced by shortened disease-free intervals and survival times (13). Spindle cell (malignant myoepithelium) and papillary adenocarcinomas were too few for any conclusions to be made.

A significant relationship between the size of the primary tumor and ER content was not found. Similarly, in one previous study of human breast cancer, no correlation between size and ER content was found (20).

It is apparent from this study that ER protein-binding activity does occur in canine mammary tumor tissue. ER-positive tumor tissue tends to be similarly distributed in human and canine mammary tissue. Although the number of samples evaluated in this study is small, the presence of ER seems to correlate to the degree of differentiation of the tumor. The evidence reported here helps to further define canine mammary cancer as a model for human breast cancer.

ACKNOWLEDGMENTS

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