Published in final edited form as:

Toxicol Lett. 2010 April 1; 193(3): 224–228. doi:10.1016/j.toxlet.2010.01.012.

Equine estrogen-induced mammary tumors in rats

Yoshinori Okamoto^a, Xiaoping Liu^a, Naomi Suzuki^a, Kanako Okamoto^a, Hyo Jeong Kim^a, Y. R. Santosh Laxmi^a, Kazutoshi Sayama^b, and Shinya Shibutani^{a,*}

^a Laboratory of Chemical Biology, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794-8651

^b Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan

Abstract

Long-term hormone replacement therapy is associated with an increased risk of breast, ovarian and endometrial cancers in women. Equine estrogens are a principal component of hormone replacement therapy; however, their tumorigenic potential toward mammary tissue and reproductive organs has not been extensively explored. A pellet containing equilin was inserted under the skin of female ACI rats and the development of mammary tumors was monitored. Histological examination revealed premalignant lesions such as apocrine metaplasia in whole-mount preparations of mammary gland from the equilin-treated rats. ACI rats given 10 mg equilin developed palpable mammary tumors at 13 weeks of treatment, and 37.5% of the rats developed mammary tumors within 15 weeks. For 2.5 mg equilin, palpable tumors were observed in 8.3% of the rats after 8 weeks' treatment; the frequency was lower than that (42.9%) observed with 2.5 mg E₂. No tumors were observed in the untreated rats. Evidently, equilin is a mammary carcinogen, and this potential may be associated with development of breast and reproductive cancers in women receiving hormone replacement therapy.

Keywords

equine estrogen; mammary tumor; hormone replacement therapy; breast cancer

1. Introduction

Hormone replacement therapy (HRT) is widely used for postmenopausal women to alleviate menopausal symptoms and to protect against osteoporosis. Premarin (Wyeth-Ayerst) is the most common drug used for HRT and consists of approximately 50% estrogens and 40% equine estrogens [equilin (EQ) and equilenin (EN)] (Bolton et al., 1998), those are not produced in human body. Long-term HRT increases the incidence of breast (Colditz et al., 1995; Chen et al., 2002; Beral et al., 2003), ovarian (Lacey et al., 2002) and endometrial cancers (Grady et al., 1995), and the risk of these cancers correlates with increasing duration of HRT (Steinberg et al., 1994; Grady et al., 1995; Chen et al., 2002; Beral et al., 2003).

*Corresponding author. Tel: 631-444-7849; fax: 631-444-3218. shinya@phram.stonybrook.edu (S. Shibutani).

Declaration of interest

None declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

It has been reported that equine estrogen metabolites react readily with DNA *in vitro*, resulting in the formation of a number of DNA adducts (Bolton et al., 1998; Shen et al., 1998). EQ and EN are metabolized by cytochrome P450 enzymes to their 4-hydroxyl and 2-hydroxyl forms (Bolton et al., 1998). 4-Hydroxyequilin (4-OHEQ) is autoxidized to an *o*-quinone which isomerizes to 4-hydroxyequilenin (4-OHEN)-*o*-quinone; therefore, 4-OHEQ and 4-OHEN produce identical dC, dA, and dG adducts in DNA (Shen et al., 1998; Zhang et al., 1999; Terashima et al., 2002). Some of the adducts were detected using LC coupled to ES tandem mass spectrometry in human breast tumor and adjacent normal tissues of several patients receiving Premarin, and in paraffin-embedded breast tumor tissues (Embrechts et al., 2003). Mutagenic events induced by 4-OHEQ were observed in a *supF* shuttle vector plasmid propagated in human cells (Yasui et al., 2003). We have reported that the dC and dA adducts are frequently miscoded during DNA synthesis catalyzed by human DNA polymerases expressed highly in mammary tissue and reproductive organs (Suzuki et al., 2004; Yasui et al., 2006 & 2007).

In addition, redox cycling between the *o*-quinone of 4-OHEN and its semiquinone radical generates superoxide, hydrogen peroxide, and ultimately reactive hydroxyl radicals (Chen et al. 1998). When 4-OHEN was incubated with DNA or breast cancer cells in culture, increased amounts of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) lesions were detected as a typical oxidative DNA damage (Han et al., 1995; Chen et al., 1998 & 2000; Zhang et al., 2001; Liu et al., 2002; Okamoto et al., 2008). Similar effects were observed in the livers and uteri of mice given EN orally (Okamoto et al., 2008) and in mammary glands of rats that received 4-OHEN injected directly into the mammary fat pads (Zhang et al., 2001). Oxidative damage may also be involved at the initiation stage of equine estrogen carcinogenesis.

Estrogen [17 β -estradiol (E₂)] (Shull et al., 1997; Turan et al., 2004) and synthetic estrogens, diethylstilbestrol (Dunning et al., 1947; Rothschild et al., 1987) and 17 α -ethinylestradiol (Holtzman et al., 1981), have shown to induce mammary tumor in August Copenhagen Irish (ACI) rats; the production of diethylstilbestrol and 17 α -ethinylestradiol were stopped in 1997 and 2004, respectively. Treatment of Premarin or a mixture of EQ and EN to male Syrian golden hamsters for 9 months resulted in 100% tumor incidence in the kidney (Li and Li 1995). However, the tumorigenic potential of equine estrogens to the targeted organs has not yet been determined. In the present study, we investigated the capability of EQ to induce mammary tumors in the ACI rat model.

2. Materials and Methods

2.1. Animals and treatments

Animals were used in compliance with guidelines established by the NIH Office of Laboratory Animal Welfare. Rats (ACI, 8-week-old females, Harlan) were given water and food *ad libitum* and kept on a 12-h light/dark cycle throughout the study. Following an established protocol for E₂ (Turan et al., 2004), after one week of acclimation, the following pellet (3 mm diameter for 90 days' release; Innovative research of America, FL) was implanted under the dorsal skin with light isoflurane anesthesia; 2.5 mg EQ (n = 16 rats), 10 mg EQ (n = 12), and the control (n = 11). ACI rats implanted a 2.5 mg E₂ pellet (n = 7) were used as a positive control. Body weight and development of mammary tumors (by palpation) were monitored once a week for 18 weeks.

2.2. Mammary whole-mount preparation and morphometric analysis

Rats (4 rats per each time point) were euthanized by CO₂ asphyxiation at 2 and 4 months after EQ implantation. Following an established protocol (Cotroneo et al., 2002; Saunier et al., 2003), the skin containing mammary glands was collected and fixed in 10% neutral buffered

formalin for at least 3 days. The mammary glands were then dissected free from skin and processed as a whole mount. The glands were defatted in ethanol, acetone, chloroform and ethanol again for at least 3 days in each solvent. After rehydration, the glands were stained with hematoxylin and washed with distilled water. The stained glands were cleaned up under a stereomicroscope, dehydrated in ethanol, cleared in xylene and mounted. Photographs were taken using a Leica DFC300FX digital color camera mounted on a Leica DM5000B microscope (Leica, Wetzlar, Germany).

2.3. Pathological determination of mammary tumor

Mammary gland including tumor was collected, fixed in 10% phosphate-buffered formalin for 16 hours. Two pieces from the middle of the formalin-fixed tumor were dehydrated and embedded individually in paraffin blocks. Paraffin-embedded mammary tumor was cut to 4- μ m thick sections and mounted on microscopic slides. The sections were deparaffinized in xylene, dehydrated in graded alcohol, and processed routinely and stained with hematoxylin and eosin (H&E). The sections were evaluated pathologically by two independent investigators under an Olympus DP20 Digital Microscope and images were captured with a 2 megapixel digital color camera (DP20, Olympus, U.S.A.).

3. Results

3.1. Growth of rats treated with EQ

The growth rate of ACI rats given a 2.5 mg EQ pellet (Fig. 1) or a 2.5 mg E₂ pellet (data not shown) was not significantly different from that of the control group during the entire experimental period (Fig. 1). However, the growth rate of rats given a 10 mg EQ pellet was significantly lower than that of the control; the body weight after the 18 weeks' treatment was approximately 20% lower than that of the control (Fig. 1).

3.2. Histological examination of mammary gland

Extension of the mammary glands, branching of the ducts, and the number of end buds and alveoli were all obviously increased by EQ treatment [2.5 mg EQ for 2 months (Fig. 2B) and 4 months (Fig. 2C)]. Moreover, premalignant lesions such as apocrine metaplasia were observed in whole-mount preparations of mammary gland from the EQ-treated rats (Figs. 2B, 2C and 2D). In contrast, neither enlargement of the mammary glands nor premalignant lesions were detected in the untreated ACI rats (Fig. 2A).

3.3. Evidence of mammary tumors

In 16 ACI rats given a 10 mg EQ pellet, palpable mammary tumors were observed in 4 rats after 13 weeks. Tumors were detected in six rats (37.5 %) within 18 weeks (Fig. 3). Among 12 ACI rats treated with a 2.5 mg EQ pellet, palpable tumors developed in only one rat (8.3 %); in contrast, among 7 rats treated with the same dose of E₂ pellet, 3 rats developed palpable tumors (42.9 %) (Fig. 3). Mammary tumors were confirmed by pathological examination (Fig. 4D). All tumors showed some characteristic morphological changes; disturbance of cellular orientation, hyperchromasia, and nuclear pleomorphisms were observed. The malignant lesions were ductal carcinomas (adenocarcinomas). No tumors were observed in the 11 non-treated rats (Figs. 4A and 4C), even after 28 weeks.

4. Discussion

Equine estrogens are widely used as a component of premarin used for HRT. The mechanism of forming DNA damage induced by equine estrogens (Bolton et al., 1998) and their mutagenic

properties (Yasui et al., 2003; Suzuki et al., 2004; Yasui et al., 2006 & 2007) have been explored. However, the tumorigenic potential of equine estrogens has not yet been determined.

Equine estrogens composed in Premarin are the 3-sulfate forms called as conjugated equine estrogen. The conjugated equine estrogens taken orally are more effectively absorbed into body through gastrointestinal, compared with the free estrogens (Schindler et al., 1982). To avoid the rapid degradation and excretion of the free form of EQ and to absorb effectively into body, subcutaneous treatment, instead of oral treatment, by implanting the pellet under the dorsal skin was used in the present study.

The growth rate of rats received a 2.5 mg EQ or E₂ pellet was not significantly different from the control, as reported earlier for a 2.0 mg E₂ pellet (Turan et al., 2004). However, when a 10 mg EQ pellet was implanted, the growth rate was significantly lower than that of the control (Fig. 1). This effect on growth was observed in ACI rats subjected to a high dose of E₂ pellet treatment (Cotroneo et al., 2002; Blank et al., 2008). Since the weight-loss observed in E₂-treated rats was due to the lowering food-intake (Redick et al., 1973), the similar effect may be involved in reducing the body weight observed in rats received a high dose of EQ.

Premarin (0.625 or 1.25 mg/day) is composed of approximately 40% equine estrogens (Bolton et al., 1998); the daily dose of equine estrogens for women (60 kg body weight) is approximately 0.25 mg (0.0042 mg/kg) or 0.5 mg (0.0083 mg/kg). Because the EQ pellet used in the present study releases 2.5 mg or 10 mg over 90 days, the ACI rats (~160 g body weight) received approximately EQ 0.056 mg (0.17 mg/kg/day) or 0.22 mg (0.69 mg/kg/day) daily. Thus, we estimate that the rats received doses at least 20 times (for the 2.5 mg pellet) or 80 times (for the 10 mg pellet) higher than the recommended dose for women. In a study that reported estrogen-induction of kidney tumors in hamsters (Li and Li 1995), a pellet containing 20 mg of equine estrogens was implanted every 2.5 months. The daily dose to the hamsters was approximately 0.27 mg, which is 4.8 and 1.2 times higher, respectively, than those for the 2.5 mg and 10 mg EQ pellets. Using the EQ doses similar to or lower than that reported for inducing kidney tumors, many premalignant lesions were observed in the mammary glands of all EQ-treated rats. Thirty-eight percent (6/16) of ACI rats implanted with a 10-mg EQ pellet developed mammary tumors within 18 weeks. With a fourfold lower dose (2.5 mg) of EQ, 8.3% (1/12) developed mammary tumors. Our results showed apparently that equine estrogen induces mammary tumors in animals.

A very low incidence (11%, 23/209 rats) of spontaneous mammary tumors was reported for the ACI strain after 3 years (Segaloff and Maxfield, 1971; Maekawa and Odashima, 1975). In our separate experiment, untreated ACI rats (n = 10) did not promote mammary tumors even after 52 weeks (data not shown). The incidence of spontaneous mammary tumor during this study may be negligible.

In a similar study with estrogens (Turan et al., 2004), 50% of ACI rats (4/8 rats) implanted with a pellet containing 3 mg E₂ developed mammary tumors after 18 weeks of treatment; the incidence of mammary tumors in rats implanted with a 1.0 mg E₂ pellet was 12.5% (1/8 rats). Consistently, with a 2.5 mg E₂ pellet 42.9 % of rats (3/7 rats) developed palpable tumors in the present study. Thus, lower doses (3.0 and 1.0 mg) of E₂ induced higher rates of mammary tumor compared to the 10- and 2.5-mg EQ doses, indicating that E₂ has a higher capability of inducing mammary tumors than EQ. Quinone formed by E₂ reacts with cellular DNA to form dG-N²- and dA-N⁶-DNA adducts, their depurinated sites, and oxidative DNA damage (Malins et al., 1993). However, the spectrum of E₂-induced DNA damage is not similar to that observed for equine estrogens; in particular, equine estrogens promote primarily unique bulky dC-DNA adducts and do not produce depurinated sites (Shen et al., 1998; Terashima et al., 2002). Therefore, the mechanism of equine estrogen-induced carcinogenesis may differ from that of

E₂-induced carcinogenesis if oxidative DNA damage does not primarily contribute to the initiation of cancer.

In conclusion, EQ used for HRT has potential to induce mammary tumors in animals, and this property may be associated with the observed high risk of developing breast and reproductive cancers in women receiving HRT. The risk of developing the cancers should be carefully weighed when equine estrogens are being considered for HRT.

Acknowledgments

This research was supported by grant ES012408 from the National Institute of Environmental Health Sciences.

References

- Blank EW, Wong PY, Lakshmanaswamy R, Guzman R, Nandi S. Both ovarian hormones estrogen and progesterone are necessary for hormonal mammary carcinogenesis in ovariectomized ACI rats. *Proc Natl Acad Sci U S A* 2008;105:3527–3532. [PubMed: 18299580]
- Bolton JL, Pisha E, Zhang F, Qiu S. Role of quinoids in estrogen carcinogenesis, *Chem. Res Toxicol* 1998;11:1113–1127.
- Beral V. Million Women Study Collaborators. Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 2003;362:419–427. [PubMed: 12927427]
- Chen Y, Shen L, Zhang F, Lau SS, van Breemen RB, Nikolic D, Bolton JL. The equine estrogen metabolite 4-hydroxyequilenin causes DNA single-strand breaks and oxidation of DNA bases in vitro, *Chem. Res Toxicol* 1998;11:1105–1111.
- Chen Y, Liu X, Pisha E, Constantinou AI, Hua Y, Shen L, van Breemen RB, Elguindi EC, Blond SY, Zhang F, Bolton JL. A metabolite of equine estrogens, 4-hydroxyequilenin, induces DNA damage and apoptosis in breast cancer cell lines, *Chem. Res Toxicol* 2000;13:342–350.
- Chen CL, Weiss NS, Newcomb P, Barlow W, White E. Hormone replacement therapy in relation to breast cancer. *JAMA* 2002;287:734–741. [PubMed: 11851540]
- Colditz GA, Hankinson SE, Hunter DJ, Willett WC, Manson JE, Stampfer MJ, Hennekens CH, Rosner B, Speizer FE. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N Engl J Med* 1995;332:1589–1593. [PubMed: 7753136]
- Cotroneo MS, Wang J, Fritz WA, Eltoum IE, Lamartiniere CA. Genistein action in the prepubertal mammary gland in a chemoprevention model. *Carcinogenesis* 2002;23:1467–1474. [PubMed: 12189189]
- Dunning WF, Curtis MR, Segaloff A. Strain differences in response to diethylstilbestrol and the induction of mammary gland, adrenal and bladder cancer in the rat. *Cancer Res* 1947;7:511–521.
- Embrechts J, Lemiére F, Van Dongen W, Esmans EL, Buytaert P, Van Marck E, Kockx M, Makar A. Detection of estrogen DNA-adducts in human breast tumor tissue and healthy tissue by combined nano LC-nano ES tandem mass spectrometry. *J Amer Soc Mass Spec* 2003;14:482–491.
- Grady D, Gebretsadik T, Kerlikowske K, Emster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. *Obst Gynec* 1995;85:304–313. [PubMed: 7824251]
- Han X, Liehr JG. Microsome-mediated 8-hydroxylation of guanine bases of DNA by steroid estrogens: correlation of DNA damage by free radicals with metabolic activation to quinones. *Carcinogenesis* 1995;16:2571–2574. [PubMed: 7586168]
- Holtzman S, Stone JP, Shellabarger CJ. Synergism of estrogens and X-rays in mammary carcinogenesis in female ACI rats. *J Natl Cancer Inst* 1981;67:455–459. [PubMed: 6943382]
- Lacey JV Jr, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, Schatzkin A, Schairer C. Menopausal hormone replacement therapy and risk of ovarian cancer. *JAMA* 2002;288:334–341. [PubMed: 12117398]
- Li JJ, Li SA, Oberley TD, Parsons JA. Carcinogenic activities of various steroidal and nonsteroidal estrogens in the hamster kidney: relation to hormonal activity and cell proliferation, *Cancer Res* 1995;55:4347–4351.

- Liu X, Yao J, Pisha E, Yang Y, Hua Y, van Breemen RB, Bolton JL. Oxidative DNA damage induced by equine estrogen metabolites: role of estrogen receptor α . *Chem. Res Toxicol* 2002;15:512–519.
- Malins DC, Holmes EH, Polissar NL, Gunselman SJ. The etiology of breast cancer. Characteristic alteration in hydroxyl radical-induced DNA base lesions during oncogenesis with potential for evaluating incidence risk. *Cancer* 1993;71:3036–3043. [PubMed: 8387875]
- Maekawa A, Odashima S. Spontaneous tumors in ACI/N rats. *J Natl Cancer Inst* 1975;55:1437–1445. [PubMed: 1206762]
- Okamoto Y, Chou PH, Kim SY, Suzuki N, Laxmi YRS, Okamoto K, Liu X, Matsuda T, Shibutani S. Oxidative DNA damage in Xpc-knockout and its wild mice treated with equine estrogen. *Chem. Res Toxicol* 2008;21:1120–1124.
- Redick JH, Nussbaum AI, Mook DG. Estradiol induced suppression of feeding in the female rat: dependence on body weight. *Physiol Behav* 1973;10:543–547. [PubMed: 4708519]
- Rothschild TC, Boylan ES, Calhoon RE, Vonderhaar BK. Transplacental effects of diethylstilbestrol on mammary development and tumorigenesis in female ACI rats. *Cancer Res* 1987;47:4508–4516. [PubMed: 3607779]
- Saunier E, Dif F, Kelly PA, Edery M. Targeted expression of the dominant-negative prolactin receptor in the mammary gland of transgenic mice results in impaired lactation. *Endocrinology* 2003;144:2669–2675. [PubMed: 12746331]
- Schindler AE, Bolt HM, Zwirner M, Hochlehnert G, Göser R. Comparative pharmacokinetics of oestradiol, oestrone, oestrone sulfate and “conjugated oestrogens” after oral administration. *Arzneimittelforschung* 1982;32:787–791. [PubMed: 6289846]
- Segaloff A, Maxfield WS. The synergism between radiation and estrogen in the production of mammary cancer in the rat. *Cancer Res* 1971;31:166–168.
- Shen L, Qiu S, Chen Y, Zhang F, van Breemen RB, Nikolic D, Bolton JL. Alkylation of 2'-deoxynucleosides and DNA by the Premarin metabolite 4-hydroxyequilenin semiquinone radical. *Chem. Res Toxicol* 1998;11:94–101.
- Shull JD, Spady TJ, Snyder MC, Johansson SL, Pennington KL. Ovary-intact but not ovariectomized female ACI rats treated with 17 β -estradiol rapidly develop mammary carcinoma. *Carcinogenesis* 1997;18:1595–1601.
- Steinberg KK, Smith SJ, Thacker SB, Stroup DF. Breast cancer risk and duration of estrogen use: The role of study design in meta-analysis. *Epidemiology* 1994;5:415–421. [PubMed: 7918811]
- Suzuki N, Yasui M, Laxmi YRS, Ohmori H, Hanaoka F, Shibutani S. Translesion synthesis past equine estrogen-derived 2'-deoxycytidine DNA adducts by human DNA polymerases η and κ . *Biochemistry* 2004;43:11312–11320. [PubMed: 15366941]
- Terashima I, Suzuki N, Shibutani S. ³²P-Postlabeling/polyacrylamide gel electrophoresis assay: application to the detection of DNA adducts. *Chem. Res Toxicol* 2002;15:305–311.
- Turan VK, Sanchez RI, Li JJ, Li SA, Reuhl KR, Thomas PE, Conney AH, Gallo MA, Kauffman FC, Mesia-Vela S. The effects of steroidal estrogens in ACI rat mammary carcinogenesis: 17 β -estradiol, 2-hydroxyestradiol, 4-hydroxyestradiol, 16 α -hydroxyestradiol, and 4-hydroxyestrone. *J Endo* 2004;183:91–99.
- Yasui M, Matsui S, Laxmi YRS, Suzuki N, Kim SY, Shibutani S, Matsuda T. Mutagenic events induced by 4-hydroxyequilin in supF shuttle vector plasmid propagated in human cells. *Carcinogenesis* 2003;24:911–917. [PubMed: 12771036]
- Yasui M, Laxmi YRS, Ananthoju SR, Suzuki N, Kim SY, Shibutani S. Translesion synthesis past equine estrogen-derived 2'-deoxyadenosine DNA adducts by human DNA polymerases η and κ . *Biochemistry* 2006;45:6187–6194. [PubMed: 16681391]
- Yasui M, Suzuki N, Liu X, Okamoto Y, Kim SY, Laxmi YRS, Shibutani S. Mechanism of translesion synthesis past an equine estrogen-DNA adduct by Y-family DNA polymerases. *J Mol Biol* 2007;371:1151–1162. [PubMed: 17603077]
- Zhang F, Chen Y, Pisha E, Shen L, Xiong Y, van Breemen RB, Bolton JL. The major metabolite of equilin, 4-hydroxyequilin, autoxidizes to an o-quinone which isomerizes to the potent cytotoxin 4-hydroxyequilenin-o-quinone. *Chem. Res Toxicol* 1999;12:204–213.

Zhang F, Swanson SM, van Breemen RB, Liu X, Yang Y, Gu C, Bolton JL. Equine estrogen metabolite 4-hydroxyequilenin induces DNA damage in the rat mammary tissues: formation of single-strand breaks, apurinic sites, stable adducts, and oxidized bases, *Chem. Res Toxicol* 2001;14:1654–1659.

Figure 1.

Body weight change of ACI rats implanted with an EQ pellet. ACI rats were implanted with an EQ [0 (n = 11), 2.5 (n = 16) or 10 (n = 12) mg] pellet and their body weight was measured once a week for 18 weeks. Data are expressed as mean values $\pm$ SD. Student *t*-test was used for statistical analysis; a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Figure 2. Morphological determination of mammary gland of EQ-treated ACI rats. Mammary gland collected from rats implanted with placebo (A) or EQ (2.5 mg) pellet for 2 months (B) and 4 months (C) was stained with hematoxylin (magnification 12.5 $\times$). Further magnification of the premalignant lesion indicated by an arrow in B (magnification 100 $\times$) (D).

Figure 3.

Cumulative incidence of mammary tumors in EQ-treated ACI rats. Development of mammary tumors in rats implanted with an EQ [0 mg (n = 11), 2.5 mg (n = 16) or 10 mg (n = 12)] or E₂ [2.5 mg (n = 7)] pellet was monitored once a week for 18 weeks.

Figure 4.

Development of mammary tumor in EQ-treated ACI rats. Mammary gland in an untreated rat (A) and an EQ-treated rat (B) was used for pathological determination (C for untreated rat and D for EQ-treated rat). The fixed mammary gland was stained with hematoxylin (magnification 40x). Sections were counterstained with H&E staining.