TITLE: Effects of Naturally-Occurring Estrogen-Fatty Acid Esters on Mammary Cell Growth and Carcinogenesis in Female Rats

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    This is the final report for the Predoctoral Taineeship Award (No. DAMD17-02-1-0567). The studies described in the original grant proposal have been completed. The major findings included the following: (i) We demonstrated, for the first time, that the naturally-occurring estrogen fatty acid esters have a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with estradiol. (ii) We showed that the endogenous estrogen fatty acid esters are pathogenically more important than estradio for the selective induction of mammary tumor formation. (iii) We found that the estrogen esterase activity is higher in the breast tissue than in the uterus, which provides the bases for our hypothesis, the endogenous estrogen-fatty acid esters are mammary-selective estrogens for stimulation of cell growth and cancer formation.

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Introduction

This is the final report for the Predoctoral Traineeship Award (No. DAMD17-02-1-0567). I like to note that this Predoctoral Traineeship Award was originally awarded to Ms. Laura H. Mills in early 2002. Around June 2003, Ms. Laura Mills was approved for graduation with a PH.D. Degree. At the request of my supervisor (Professor Bao Ting Zhu), I was named as the replacement P.I. on this grant proposal shortly following Ms. Mills' departure from the project.

While serving as the replacement P.I., I helped to conduct additional studies required for the completion of this project and I have also helped with the analysis of some of the data originally obtained by Ms. Mills. Now the studies described in the original grant proposal by Ms. Laura Hook Mills have been completed.

Body

The Specific Aims of the Original Proposal:

Aim 1: I will evaluate the stimulatory effects of E2-17β-stearate and 4-hydroxyestradiol-17β-stearate, two representative estrogen-fatty acid esters, on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. Their effects will be compared with the effects of unesterified E2 or 4-hydroxyestradiol. The circulating levels of prolactin, FSH, and LH will also be determined in all animals, which will serve as an indicator of estrogen's action on the pituitary. During these animal experiments, I will also collect the pituitary, thymus, and liver, and if time allows, and the growth-stimulatory effects of esterified vs. unesterified estrogens in these organs will be determined and compared with their effects in the fat-rich mammary tissue.

Aim 2: I will evaluate the carcinogenic activity of E2-17β-stearate and 4-hydroxyestradiol-17β-stearate on mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats. The results will be compared and correlated with the growth stimulatory effects determined under Aim 1.

Aim 3: I will evaluate the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue, and its activity will be compared with that found in other organs such as the uterus and liver. These analyses will provide insights into our understanding of the mechanisms underlying a preferential mitogenic action (and perhaps carcinogenic action) of estrogen-fatty acid esters in the breast over the uterus.
Major findings by Ms. Laura H. Mills (the original P.I.) and me (the replacement P.I.).

1. We have completed evaluating the stimulatory effects of E$_2$-17β-stearate on the growth of mammary vs. uterine cells in ovariectomized female Sprague-Dawley rats, and their effects were compared with the effects of unesterified E$_2$. Experimentally, 0.5 or 5 nmol of E$_2$-17β-stearate or E$_2$ was released daily to ovariectomized female rats through an Alzet pump implanted under the back skin of the animal for 10 or 23 days. The growth-stimulatory effect of E$_2$-17β-stearate and E$_2$ on mammary glandular cells was determined according to 5-BrdU indices, and their effect on the uterus was determined by measuring both the 5-BrdU-labeling index and the uterine wet weight. We found that chronic treatment of ovariectomized female rats with 0.5 or 5 nmol/day of E$_2$-17β-stearate for 10 or 23 days had a stronger stimulatory effect on mammary glandular cell proliferation than treatment with equimolar doses of E$_2$. In the uterus, however, E$_2$ was more active in stimulating the proliferation of uterine endometrial cells than E$_2$-17β-stearate at equimolar doses. Our results demonstrated, for the first time, that a naturally-occurring E$_2$-17β-fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with E$_2$.

2. We have also determined the plasma levels of prolactin (PRL), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in ovariectomized female rats treated with E$_2$-17β-stearate or E$_2$. We found that chronic administration of E$_2$ showed a stronger negative regulatory effect on the plasma LH levels than E$_2$-17β-stearate. The plasma levels of FSH in ovariectomized female rats were not significantly affected by treatment with E$_2$-17β-stearate or E$_2$. Chronic administration of E$_2$-17β-stearate or E$_2$ markedly increased the plasma PRL levels, but their effects were not significantly different from each other.

3. We have also evaluated the activity of the estrogen-fatty acid ester with E$_2$ for the induction of mammary vs. pituitary tumors in intact female ACI rats. Experimentally, the animals received an s.c. implantation of a 20 mg pellet (containing 18 μmol of an estrogen plus cholesterol or 100% cholesterol as the control) at 62 ± 3 days of their age, and the animals were monitored for a total period of 8 months. Among the 25 control animals, none developed mammary tumor and all lived healthily during the whole carcinogenesis experiment. Among the 26 animals receiving an E$_2$ pellet, all the animals developed a large pituitary tumor (average weight = 254 ± 83 mg), but only 8 of them developed mammary tumor(s). A majority of the animals died or had to be terminated early because of severe sickness, likely due to the presence of the large pituitary tumor. Among the 26 animals implanted with the same molar dose of an E$_2$-fatty acid ester preparation (containing 63% E$_2$-17β-stearate and 37% E$_2$-17β-palmitate), a total of 9 animals developed mammary tumor, and none of the animals died of non-breast cancer-related sickness. Only 3 animals (11.5%; all with mammary tumor) developed a pituitary tumor, with a slightly smaller average size (173 ± 75 mg). In conclusion, while E$_2$ was far stronger in stimulating the formation of pituitary tumor (100% incidence) than mammary tumor (30.7% incidence), E$_2$-fatty acid esters had a somewhat stronger activity than E$_2$ in inducing the mammary tumor but only had very weak activity in inducing pituitary tumor. Our data suggest that the endogenous E$_2$-fatty acid esters are pathogenically more important than E$_2$ for the selective induction of mammary tumor formation.
4. We have evaluated the activity of estrogen esterase (the enzyme that hydrolyzes estrogen-fatty acid esters to release bioactive estrogens) in the mammary tissue. We found that this esterase activity is higher in the breast tissue than in the uterus. This is a very interesting novel finding, which provided the basis for our hypothesis the endogenous estrogen-fatty acid esters are mammary-selective estrogens for the induction of cell growth and cancer formation.

Key Research Accomplishments

1. We demonstrated, for the first time, that a naturally-occurring estrogen fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with E2.

2. We showed that the endogenous estrogen fatty acid esters are pathogenically more important than E2 for the selective induction of mammary tumor formation.

3. We found that the estrogen esterase activity is higher in the breast tissue than in the uterus, which provides the basis for our hypothesis the endogenous estrogen-fatty acid esters are mammary-selective estrogens for stimulating cell growth and cancer formation.

Reportable Outcomes

Listed below are papers and abstracts that have come out of this award, with the P.I.'s names highlighted.


Listed below are some other papers published by the replacement P.I. during his graduate studies under the supervision of Dr. B. T. Zhu:


Lee WJ and Zhu BT [2005] Inhibition of enzymatic DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis [August 4, 2005; Epub ahead of print] [in press].


Conclusions

The naturally-occurring estrogen fatty acid esters have a preferential mitogenic effect in the fat-rich mammary tissues compared to estradiol. Also, these endogenous estrogen fatty acid esters are pathogenically more important than estradiol for the induction of mammary tumor formation in the animal model.
References


Lee WJ and Zhu BT [2005] Inhibition of enzymatic DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols. Carcinogenesis [August 4, 2005; Epub ahead of print] [in press].


**Appendices**

Not included.