Effects of Continuous Combined Oral Contraceptives on Mouse Mammary Gland Structure and Tumor Progression

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Abstract

The use of cyclic oral contraceptives (OC) is associated with an overall increased risk of ER+ve breast cancer by 40% (for review of OC) and of breast cancer by as much as 80% (after 10 years of OC use) (reviewed in [1]). In contrast to OCs effects on endometrial and ovarian cancer risk, OC usage has not been shown to be a preventive agent for breast cancer. Although studies involving animals vary widely, a meta-analysis calculates that overall, OC usage increases breast cancer risk by 1.24 for current, 1.19 for previous, and 1.07 for previous and current users (2). For current users, risk is further increased when the age at which the use was less than 35 years. Suggesting that there may be OC-sensitive developmental window soon after the onset of puberty and that risk is determined for a cyclic-dose regimen (i.e., those weeks on, followed by one week of hormone withdrawal). The effects of recently FDA/approved continuous OC dosing regimen on breast cancer risk has not been evaluated. Prior to its approval for general use in 2007, continuous OC was limited primarily to those needing emergency contraception, due to its increased efficacy in pregnancy prevention and completion of menstrual cycles. Continuous OC usage is associated with decreased risk in women with smoking habit and increased breast cancer risk in women taking estrogen replacement therapy (reviewed in [3]).

It has been suggested that estrogens disrupt regular cycling decreasing breast tissue estrogen levels (3). Possible due to the lack of estrogen production in the women taking OCs, which may cause increased proliferation of mammary gland cells. Typically, women on continuous OC usage are asked to cycle every 21 days, thus OC usage decreases breast cancer risk in women taking estrogen replacement therapy (reviewed in [4]). A recent study using a larger sample size showed that women taking continuous OCs had lower breast cancer risk than women taking cyclic OCs (5).

Materials and Methods

Animals
BALB/c mice were purchased from Jackson Labs LEMS 38 days of age. Mice were initiated at 4.5 g and continued on a 12:12 h light:dark cycle, with single sex, non-related mice per cage. Animals were allowed ad libitum food and water, with mice randomized into either continuous or 28-day, as a control group (three OC days, followed by one OC free day). After 28 days, 15 mice were randomized to 2 OC groups, stained with hematoxylin and eosin, and assessed for mammary gland development.

Breast cancer was assessed by inoculating the tumors into the mammary gland of C57BL/6 mice (n=6). Tumors were stained with hematoxylin and eosin, and assessed for mammary gland development.

Dietary model of OC exposure
Mice were grouped based on age, sex, and CT exposure. Both exposure groups were exposed to the same diet, (C) exposed to continuous OC (n=15), and 28 days of cyclic (n=15). Mice were implanted in the mammary gland of C57BL/6 mice (n=6). Tumors were stained with hematoxylin and eosin, and assessed for mammary gland development.

Results

Figure 1: Effect of OC on epithelial proliferation
Top: whole mounts of mammary gland images showing terminal structures in mammary glands without tumors. Fetal mammary ducts (TD) single small ducts with thin terminal branches, terminal buds (TB): ducts anastomosed to alveoli, alveolar ducts (AD): two or more clustered ducts with thin terminal branches.

Figure 2: Effect of OC on epithelial density
Epithelial density images showing terminal structures in mammary glands without tumors. Fetal mammary ducts (TD) single small ducts with thin terminal branches, terminal buds (TB): ducts anastomosed to alveoli, alveolar ducts (AD): two or more clustered ducts with thin terminal branches.

Table 1: Effects of OC on TM2H mammary tumor latency, survival, and multiplicity
Continuous OC significantly increased tumor multiplicity (time from injection to first palpable tumor) compared to control. No effect on survival (time from injection to sacrifice when tumor diameter greater than 1 cm) was seen. Palpable tumor volume was decreased by both OC groups at 7, 14, and 21. Growth rate was not significantly altered by OC; however, continuous OC caused a trend to increase growth rate. Data reported means ± SEM (n=20 (control); 20 (continuous OC) or 20 (cyclic OC). Odds ratio indicates significant differences (P<0.05).

Discussion
The results of this study suggest that OC usage can increase the risk of breast cancer. The observed increase in breast cancer risk may be due to the hormonal effects of continuous OC usage, which may cause increased proliferation of mammary gland cells. Further studies are needed to confirm these findings and to determine the long-term effects of continuous OC usage on breast cancer risk.

References