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Metabolism of Estrone Sulfate by Normal Breast Tissue: Influence of Menopausal Status and Oral Contraceptives

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The metabolism of [^{3}H]estrone sulfate ([^{3}H]E_1S) was studied in normal breast tissue from 10 premenopausal women without oral contraceptives (OC), in 12 OC users and in 9 untreated postmenopausal women. [^{3}H]E_1S was converted into estrone ([^{3}H]E_1) and estradiol-17β ([^{3}H]E_2) by tissue samples from all three groups of women, with only minor formation of other unconjugated compounds. The rate of [^{3}H]E_2 formation was significantly higher in premenopausal women without OC than in postmenopausal women. Among premenopausal women, OC users had a significantly lower rate of total hydrolysis and of [^{3}H]E_1 formation than non-users. The rate of total hydrolysis of [^{3}H]E_1S in normal breast tissue from all three groups of women was similar to that in muscle, but the rate of [^{3}H]E_2 formation was ten times higher. Both total hydrolysis rate and rate of [^{3}H]E_2 formation were significantly lower in normal breast tissue than in breast carcinoma and in normal and neoplastic endometrium. The specific ability of normal breast tissue to convert E_1S into the terminal biologically active estrogen E_2 may be important for estrogenic stimulation of the breast in subjects with low circulating E_2 levels. The lower rate of E_1 formation in OC users may reflect an inhibitory effect of the progestagen compound in such preparations.

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INTRODUCTION

There is a considerable evidence that estrogens are involved in the development and growth of breast cancer. The possibility of an increased breast cancer risk during combined estrogen/progestogen therapy, i.e. for contraception and postmenopausal replacement has been discussed, but experimental and epidemiologic studies have yielded conflicting results [1–5]. Currently much basal knowledge about estrogen metabolism and regulation of proliferation in normal breast tissue is lacking.

Estrone sulfate (E_1S) is by far the most abundant estrogen in the peripheral circulation. Although it does not bind to the estrogen receptor, E_1S can be converted into the terminal biologically active estrogen estradiol-17β (E_2) and thus exert biological activity. Conversion of E_1S into E_2 was shown independently by Vignon *et al.* [6] in MCF-7 breast cancer cells and by our group [7] in homogenates of breast carcinoma tissue. The conversion has been demonstrated in a large number of tissues, including breast carcinoma, endometrium and endometriotic tissue [8, 10]. Studies on breast cancer tissue have unequivocally shown that formation of E_2 from E_1S is far more important than intratumoral aromatization [11, 12].

In contrast to breast cancer, data on E_1S metabolism in normal breast tissue are scarce. Naitoh *et al.* [13] reported a considerably lower rate of E_2 formation from E_1S in normal breast tissue than in breast cancer in a limited material of 5 patients. The formation of E_2 in normal breast tissue may be important for the local estrogenic mileu and thus for the growth and development of an early breast cancer.

While the protective effect of combined oral contraceptives (OC) against endometrial carcinoma is well known, this is obviously not the case for breast cancer [14]. There are indications that OC as well as pure gestagens stimulate cell proliferation in normal and neoplastic breast, in contrast to the situation in the endometrium.

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Anderson *et al.* [2] demonstrated that both combined and gestagen only OCs enhanced breast epithelial cell proliferation in women <34 years. Furthermore, *in vitro* studies on human breast cancer cells have demonstrated that gestagens increase thymidine kinase activity, a well known marker of proliferation, and also increase insulin receptor content and insulin mediated cell growth [15, 16]. Therefore, a group of OC-treated women were included in the study.

In the present study the metabolism of E_1S was studied in normal breast tissue obtained from pre- and postmenopausal women and from women during OC-treatment. The results were also compared with previous data from other tissues including muscle, mammary carcinoma and normal atrophic and neoplastic endometrium.

EXPERIMENTAL

Clinical material

E_1S metabolism in normal breast tissue was studied in 9 postmenopausal women aged 58–67 years (mean age 62 ± 1.1 SEM) and 22 women of fertile age 18–34 years (mean age 27 ± 1.1), undergoing reduction mammoplasty for inconvenient breast size or cosmetic reasons, or undergoing surgery for benign breast disease. In the latter cases, normal tissue was taken at least 2 cm from the benign lesion. Twelve of the women of fertile age were taking combined OC, otherwise the study population was medicine free. Immediately following surgical removal, the tissue samples were washed with saline and frozen at −70°C until analyzed. Venous blood samples were drawn on the day of surgery in all the women who were not taking OC.

Methodology

The metabolism of E_1S in total homogenates of breast tissue was studied as described previously for breast cancer and for endometrium [7, 9]. Briefly, the method was as follows: Thawed tissue was homogenized with 0.06 M Tris–HCl pH 7.0. The homogenate was incubated at two different incubation times (15 and 60 min) with [^{3}H]E_1S in the presence of NADH and NADPH. The reaction was terminated by addition of unlabeled estrone (E_1) and E_2 in ethanol together with 0.5 M sodium phosphate pH 7.0. The liberated unconjugated [^{3}H]estrogens were extracted with toluene. One aliquot of the toluene phase was taken to direct liquid scintillation counting in order to determine the rate of total hydrolysis, i.e. total liberated ^{3}H radioactivity. The remaining toluene phase was evaporated and subjected to thin layer chromatography and liquid scintillation counting for the determination of the rate of formation of individual [^{3}H]estrogens.

Appropriate blank corrections were undertaken in all experiments. The assay was linear with respect to time and to the amount of enzyme source for the total hydrolysis as well as for the formation of [^{3}H]E_1 and of [^{3}H]E_2, respectively. The values were expressed as fmol of liberated unconjugated [^{3}H]estrogen per minute and per mg of protein.

Serum concentrations of E_2, total E_1 (tE_1, sum of free + conjugated E_1, ⩾85% estrone sulfate) and progesterone were analyzed by radioimmunological methods, the details of which have been given in previous communications [9, 17, 18].

Statistical methods

Statistical analysis was performed using Mann-Whitney U-test and Spearman's rank correlation test.

RESULTS

E_1 and E_2 were the main unconjugated compounds formed from E_1S by breast tissue and only minor amounts of other unconjugated compounds were formed. Median values and range for the total estrone sulfatase activity and for the formation of [^{3}H]E_2 from [^{3}H]E_1S are given in Table 1a. The rate of formation of [^{3}H]E_2 was significantly higher ($P < 0.05$) in the premenopausal women without OC than in postmenopausal women. The rates of total hydrolysis and of formation of [^{3}H]E_1, were higher in premenopausal women without OC than in OC users ($P < 0.05$). There were no differences between the groups in the ratio of formed [^{3}H]E_2 to [^{3}H]E_1. The rate of formation of [^{3}H]E_2 showed a significant positive correlation to the total hydrolysis rate in healthy postmenopausal women ($r_s = 0.73$, $P < 0.05$) and in OC users ($r_s = 0.67$, $P < 0.05$). Significant positive correlations ($r_s = 0.77$, $P < 0.05$) were found between the rates of total

Table 1a. Total hydrolysis and formation of [^{3}H]E_1 and [^{3}H]E_2 from [^{3}H]E_1S by human normal breast tissue in vitro

	(A) Premenopausal without OC $N = 10$	(B) Premenopausal with OC $N = 12$	(C) Postmenopausal healthy $N = 9$	Significant differences
Total hydrolysis	137.4 (30.7–274.8)	72.7 (6.3–241.3)	85.1 (1–184.9)	A vs B, $P < 0.05$* A vs C, NS
E_1 formation	131.7 (22.0–265.2)	68.8 (6.2–229.8)	83.1 (<0.01–182.7)	A vs B, $P < 0.05$* A vs C, NS
E_2 formation	2.2 (1.2–3.4)	1.5 (<0.01–6.4)	1.2 (<0.01–2.6)	A vs B, $P = 0.075$, NS A vs C, $P < 0.05$*

Values are median and range in fmol × mg protein^{-1} × min^{-1}

Table 1b. Total hydrolysis and formation of [^{3}H]E_2 from [^{3}H]E_1S by different tissues in earlier studies from our group [7, 19, 20]

	(D) Postmenopausal breast cancer $N = 20$	(E) Postmenopausal atrophic endometrium $N = 9$	(F) Postmenopausal endometrial carcinoma $N = 7$	(G) M. rectus abdominis & M. pectoralis $N = 9$	Significant differences
Total hydrolysis	285.9 (86.9–887.1)	196 (5.2–1064)	785 (522–2147)	69.3 (25.6–188.7)	C vs D, $P = 0.0002$*** C vs E, $P = 0.046$* C vs F, $P = 0.0005$*** C vs G, NS A vs G, NS B vs G, NS
E_2 formation	6.2 (0.4–110.3)	2.9 (<0.01–15.2)	43.5 (5.3–249.3)	0 (0–0.4)	C vs D, $P = 0.001$** C vs E, NS C vs F, $P = 0.0005$*** C vs G, $P = 0.0011$** A vs G, $P = 0.0002$*** B vs G, $P = 0.0002$***

Values are median and range in fmol × mg protein^{-1} × min^{-1}.

hydrolysis and of E_1 formation and serum progesterone values in women without OC.

The abovementioned figures from normal breast tissue were compared with the corresponding activities in muscle, breast cancer and normal atrophic and neoplastic endometrium obtained in postmenopausal women in previous studies from our group using identical technique as in our present study [7, 19, 20]. The data for these tissues are given in Table 1b. The total hydrolysis rate in normal breast tissue was significantly lower than in breast cancer and normal atrophic and neoplastic endometrium. The rate of formation of E_2 was significantly higher in all three groups of normal breast tissue than in muscle but significantly lower than in breast cancer and in neoplastic endometrium.

DISCUSSION

The breast is a target organ for sex hormone action and the difference in hormone sensitivity from non-target tissues such as muscle is well known. While the rate of total hydrolysis was about the same in muscle as in normal breast tissue the rate of formation of E_2 was about 10 times higher in normal breast tissue, indicating a biological significance of this transformation. Intracellular formation of E_2 from E_1S may thus play an important role for the local estrogenic milieu in subjects with low peripheral concentrations of E_2. Our results further confirm previous findings of Naitoh *et al.* [13] of a higher enzyme activity in neoplastic breast tissue. The reason for this difference is not known, but autocrine effects of growth factors may be involved [21].

In general, E_1S conversion rates were lower in normal breast tissue from postmenopausal and OC-treated women than in untreated premenopausal subjects, although the differences reached statistical significance only for the E_2 formation in postmenopausal women and for total hydrolysis and formation of E_1 in the OC-users. The lower rate of E_2 formation in the postmenopausal women might be an age-related phenomenon: An age related decrease in the formation of E_2 as well as in 17-beta reduction of E_1 and of 4-androstene-3,17-dione in breast cancer tissue has earlier been demonstrated by our group and by Varela and Dao [7, 22].

However, age-related changes in enzyme activity can hardly explain the lower rate of E_2 formation in the OC-treated women. Prost-Avalet *et al.* [23] have demonstrated that certain synthetic progestagens inhibit estrone sulfatase activity in human breast carcinoma tissue, while natural progesterone stimulates this activity. The lower enzyme activity in OC-treated women may therefore be ascribed an inhibitory effect of the 19-nor progestagen component of the OC. The reported proliferative effects of combined OC as well as of pure progestagen preparations on breast tissue [2, 15, 16] are therefore probably not mediated via an increased intracellular formation of E_2 but may instead be mediated via a direct stimulation of the progesterone receptor (PgR). We have recently shown that the PgR in breast epithelial cells from healthy volunteers with normal ovulatory cycles, in contrast to the endometrial PgR, is not down regulated during the luteal phase [24]. This may be one possible explanation for the reported maximal cell proliferation during the luteal phase of the menstrual cycle [1, 2].

To conclude, normal breast tissue has a significant ability to convert E_1S into the terminal biologically active estrogen E_2. This may be important for the estrogenic stimulation of the breast in subjects with low circulating E_2 levels. Our results indicate that OC treatment does not increase E_2 formation in normal breast tissue. The reported proliferative effects of OC treatment and gestagens may therefore be a direct progestagen effect, possibly mediated via the PgR. Further studies by our group will evaluate the effects of OC treatment on steroid receptors and proliferation in breast cells from healthy women.

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