Estradiol Synthesis and Metabolism and Risk of Ovarian Cancer in Older Women Taking Prescribed or Plant-derived Estrogen Supplementation

Linda S M Gulliver*
Faculty of Medicine, University of Otago, Dunedin, New Zealand

Abstract

Estradiol, the most potent of the biological estrogens, is implicated in the genesis of ovarian epithelial cancer, a heterogeneous cancer affecting mainly older women. The postmenopausal ovary traditionally has not been viewed as contributing significantly to estradiol synthesis, since this is thought to occur almost exclusively as the result of peripheral aromatization of adrenal androgens. Recent evidence supports a role for both normal and malignant ovarian tissue in *de novo* synthesis of estradiol using inactive biological precursors and available enzymatic pathways. The process is termed “intracrinology”. The present paper reviews available evidence for the intracrinological synthesis of estradiol in ovarian surface epithelium. It further proposes how exogenous supplementation with synthetic hormone replacement may act to augment this process by increasing the risk of developing ovarian epithelial cancer in older women. Phytoestrogens are also examined for their role in regulating levels of estradiol metabolites with potent estrogenic and carcinogenic potential.

Keywords: Estradiol intracrinology synthesis/metabolism; Estrogen supplementation; Ovarian epithelial cancer; Older ovary; Ovarian surface epithelium

Abbreviations: CYP: Cytochrome P450 Enzyme; HSD: Hydroxysteroid Dehydrogenase Enzyme; E1S: Estrone Sulfate; DHEA-S: Dehydroepiandosterone – sulfate; STS: Steroid Sulfatasae; HGSC: High Grade Serous Carcinoma; RT-PCR: Reverse Transcription Polymerase Chain Reaction; OSE: Ovarian Surface Epithelium; EST: Estrogen Receptor; SHBG: Sex Hormone Binding Globulin; COMT: Catechol-O-Methyl Transferase; OVCAR-3: Ovarian Cancer 3 Cell Line; P13: Phosphatidyl-inositol 3; AKT: Protein kinase B; NFxB: Nuclear Factor Kappa B; ER: Estrogen Receptor

Estradiol Synthesis in the Premenopausal Ovary

All steroid hormones, of which estradiol is an example, are lipids that have cholesterol as their common precursor substrate. The synthesis of sex steroid hormones from cholesterol involves a series of sequential steps that result in the cleavage of side-chains, reorganization of olefinic bonds, and the addition of hydroxyl groups. For estradiol synthesis, this pathway is from cholesterol to pregnanes, androstanediol, androstenedione, androstenedione and requires the actions of 17β HSD1 in converting androstenedione to testosterone, after which aromatase completes the conversion through aromatase 17β [1]. This pathway is subsequently termed “the aromatase pathway” for estradiol synthesis. In the luteal phase of the ovulatory cycle, mammalian theca and cells of the corpora lutea (theca lutein) act together with granulosa lutein cells of corpora lutea, to produce sizeable amounts of both estradiol and progesterone. Throughout the cycle, estrone-sulphate (E1S) and dehydroepiandosterone sulfate (DHEA-S - available in small amounts in the premenopausal ovary) may also undergo conversion to estrone and DHEA respectively via the sulfatases (STS). This is “the sulfatase pathway” for estradiol synthesis, since the actions of the sulfatases allows the final conversion through to estradiol to be completed by HSDs 3 and 17 and P450arom.

The localization and expression of the major enzymes required for ovarian steroidogenesis are shown in figure 1.

To summarize, the availability of cholesterol, the relative amounts and type of enzymes in each tissue or cell compartment, and the actions of FSH on granulosa cell [3] are the three variables upon which estradiol synthesis in the premenopausal mammalian ovary depends.

Estradiol and the Postmenopausal Ovary: Potential for Oncogenesis

Following menopause, peripheral estradiol levels in the blood are thought to be mainly due to contribution from the adrenals and peripheral aromatization of androgens to estrogen in adipose tissue and skin, where aromatase activity correlates with estradiol production. There is evidence however, that the postmenopausal ovary retains the ability to produce both androgens [4] and estrogen [5,6].

The degree to which ovarian surface epithelium (OSE) is capable of...
Conjugation of estrone to a sulphur group producing estrone sulfate (EST) compared to younger mice. EST, an enzyme that catalyzes the conversion of estrone to estradiol, also expresses mRNA for 17βHSD reductase required for the synthesis of estradiol from estrone [26]. In confluent culture, normal OSE from pre and postmenopausal women has been shown capable of de novo synthesis of both estrogen and progesterone using HSDs [27]. Thus, an increase in the STS to EST ratio may potentially boost local ovarian production of estradiol 17β, the far more potent biologically active estrogen that binds with strong affinity to its receptor. A current review targeting sulfatase activity for estrogen production in OSE supports this view [28] and underpins the emerging significance of the sulfatase pathway for generating potentially damaging levels of estradiol in OSE, conceivably predisposing it to oncogenesis.

It is possible that ovarian aging may have an effect on the stability of EST proteins. This may confer reduced ability to sulfonate estrone and estradiol obtained from peripheral blood via organic anion transporting polypeptides [28], which in turn may lead to increased levels of unconjugated estradiol in the ovary. Indeed in a recent study completed by our own lab, we found using radioimmunoassay, that some older mice had elevated levels of endogenous estradiol in ovarian tissue (>300 pg/mL; normal range 66.2-117 pg/mL). Furthermore, mice administered exogenous estradiol continued to show significantly elevated estradiol levels in ovarian tissue 2 weeks following cessation of estrogen treatment, indicating that unconjugated estradiol accumulates and is retained in ovarian tissue [29,30].

These findings may go some way to providing an explanation as to why older women taking exogenous estradiol in the form of hormonal replacement therapy carry greater risk for the development of ovarian epithelial cancer. Friel et al. [31] reported that an oral estradiol dose regimen of between 1-2 mg/day can lead to a condition of estradiol overdose - as evidenced by urinary excretion of estrone 5-10 times the upper limit of the reference range for premenopausal women. Of note is that the present recommended estradiol dose in the U.S remains anywhere between 0.45 mg/day–2 mg/day.

Phytoestrogen Intake and Risk of Ovarian Epithelial Cancer

While ample epidemiological evidence exists for hormone replacement therapies as having a role in the development of ovarian...
cancers, some evidence suggests that phytoestrogens taken in the diet may actually have the opposite effect. Phytoestrogens which include isoflavones (mostly soy-derived) and lignans (derived from grains, seeds, vegetables, fruits and berries) are established as having both estrogenic and anti-estrogenic properties [32,33]. Phytoestrogen consumption has been reported to either confer significantly reduced risk of ovarian cancer [34-36] show a non-significant inverse association with ovarian epithelial cancer [37], or show no association with overall ovarian epithelial cancer risk [38]. Interestingly in the latter prospective cohort study, fiber intake was associated with a decreased risk of borderline, but not invasive ovarian epithelial cancer [38]. Re-uptake of estradiol metabolites found in bile is slowed by the presence of fiber in the intestine, lowering total estrogen. This mechanism for regulating estradiol may have little effect on established tumors capable of autonomous endocrine activity.

The differing results from the above studies may be a consequence of their differing methodologies (case-control based or prospective cohorts, dietary-derived only phytoestrogens versus dietary and supplement derived), different sample populations, age and menopausal status of the women. Moreover, ovarian cancers are highly heterogeneous and this has received little attention in the design and interpretation of these studies. Perhaps importantly, authors of the recent Swedish study [38] (where no association was seen between phytoestrogen intake and ovarian cancer risk) reported that overall bean/soy consumption in their cohort was low. Since other studies [34-37] were able to show a dose-response correlation, whereby subjects making up the highest quintile of phytoestrogen consumption showed reduced risk for ovarian cancer compared to those in the lowest quintile, the normally low intake of phytoestrogens in the Swedish cohort may have influenced results from that study. Studies which report changes to estradiol metabolism that reduce risk for developing ovarian cancer when women ingest higher rather than lower quantities of phytoestrogens in the form of flaxseed (a lignan) and isoflavones [39,40], lend further support for a protective role for phytoestrogens in the development of ovarian cancer.

There is an important emerging body of research investigating how ingested phytoestrogens act to control endogenous levels of estradiol by controlling the activity of estradiol’s active metabolites. Circulating estradiol in human blood is controlled by regulatory mechanisms that act independently of the well-known endocrine feedback loops, including activin and inhibin. In humans, levels of Sex Hormone Binding Globulin (SHBG) act to bind and therefore control to some extent the actions of free unbound estradiol. Levels of SHBG increase with increasing levels of circulating estradiol and reflect the fact that more hormone is available for binding. The half-life of estradiol in the postmenopausal female is around 3 hours [41] with significant more hormone is available for binding. The half-life of estradiol in circulating SHBG in premenopausal women [47]. Decreases in 16a-estradiol, 4-hydroxyestrone and 4-hydroxyestradiol have also been shown in premenopausal women with high soy isoflavone intakes of 130 mg/day compared to those with low intakes of 7-10 mg/day [40]. Moreover, the daily ingestion of large amounts (10 g) of ground flaxseed over a period of seven weeks in postmenopausal women has been reported to dramatically induce 2-hydroxylation of estrone and improve the ratio of 2/16α-estradiol, whereas only moderate effects were observed with reduced intakes of 5 g flaxseed per day [39]. Interestingly, supplementation with flaxseed lignans appears superior to soy in altering estrogen metabolism in postmenopausal women with respect to increasing 2α to 16α-hydroxylation. Flaxseed also moderately inhibits Cytochrome P450 arom [48,49] and modulates the activity of the 17-HSD [50].

A diet that is high in soy isoflavones increases the 2α-hydroxyestrone to 16α-hydroxyestrone ratio and lowers mid-cycle gonadotropin levels, leading to decreases in circulating estradiol, progesterone and SHBG in premenopausal women [47]. Decreases in 16a-estradiol, 4-hydroxyestrone and 4-hydroxyestradiol have also been shown in premenopausal women with high soy isoflavone intakes of 130 mg/day compared to those with low intakes of 7-10 mg/day [40]. Moreover, the daily ingestion of large amounts (10 g) of ground flaxseed over a period of seven weeks in postmenopausal women has been reported to dramatically induce 2-hydroxylation of estrone and improve the ratio of 2/16α-estradiol, whereas only moderate effects were observed with reduced intakes of 5 g flaxseed per day [39]. Interestingly, supplementation with flaxseed lignans appears superior to soy in altering estrogen metabolism in postmenopausal women with respect to increasing 2α to 16α-hydroxylation. Flaxseed also moderately inhibits Cytochrome P450 arom [48,49] and modulates the activity of the 17-HSD [50].

Some studies investigating estrogen metabolism and breast cancer have shown that estradiol metabolism favoring formation of 2-α hydroxylation over 16-α-hydroxylation decreases the risk for developing breast cancer [51,52], although other studies show mixed results [53]. The differences in results may relate to the varying methodologies employed by researchers, but may also relate to menopausal status in the women studied. Recently, in an in vitro study using ovarian cancer cell line OVCAR-3 to examine the effects of the metabolites of estradiol on proliferation and apoptosis in comparison to estradiol itself, the 17β proliferative and anti-apoptotic activity of the 16a-hydroxylated estrone was shown to outstrip that of estradiol. Surprisingly 4α-hydroxyestrone gave similar results to 17β estradiol at physiologic concentrations [54], and may exert its effects through the PI3K/Akt signaling pathway to promote ovarian carcinogenesis. Importantly, the 2α-hydroxyestrone metabolite was shown to have little activity. Although there is more research needed, these results indicate that the maintenance of pro-oncogenic to anti-oncogenic estradiol metabolites in the ovary may prove to be a very important factor in the genesis of ovarian epithelial cancer.

Finally, many phytoestrogens are known to bind both functional estrogen receptor subtypes (ERα and ERβ), and are capable of inducing transcription of estrogen responsive target genes in a dose-dependent manner [55-58]. Whether phytoestrogen binding to ER produces
the same or opposite effect to estradiol appears to depend on several factors: the type and amount of phytoestrogen [55-60], its relative binding affinity for the receptor subtype [56,57,61], the abundance from tissue to tissue of one ER subtype relative to the other [62], the presence of low-affinity (type II) nuclear binding sites [63], the ability of the phytoestrogen to utilize other non-genomic modalities (such as akt phosphorylation and NFκB) to modulate estrogenic and carcinogenic effects [64,65], and the presence of endogenous estrogen [66]. Activation of ERα by estradiol induces marked proliferation in normal and cancerous ovarian epithelial cells in vitro and in vivo [29,67-69], whereas activation of ERβ opposes the proliferative effects of ERα and has pro-apoptotic and anti-tumoral effects [69-71].

Phytoestrogens are known to bind ER with much lower affinity than estradiol [60,61], and preferentially bind ERβ [72]. Moreover, they induce the transcription of estrogen-responsive target genes to a much greater degree when bound to ERβ, rather than when bound to ERα [66]. Phytoestrogens are also capable of inducing ER-mediated gene transcription to higher levels than estradiol itself [66]. Taken together, it may be proposed that in tissues such as ovary where ERβ is abundantly expressed, phytoestrogens may act to augment the anti-carcinogenic effects of that receptor subtype. However, it should be noted that some phytoestrogens (e.g. genistein and resveratrol) have been known to act synergistically with estradiol in MCF-7 breast cancer cells [66,73], and can act as ‘super agonists’ that bind ERα as well as ERβ. It is therefore important that further research defines the effects of different phytoestrogens on the ovary, and elucidates the cellular and molecular basis for their action.

Conclusion

At this time there appears to be a paucity of both in vitro and in vivo data for estradiol intracrinology and metabolite activity in the older female, and in the development of ovarian epithelial cancer. Since long-term exposure to estradiol is an established risk factor for ovarian cancer, this is an area of research that requires much more attention.

References


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