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REGULATION OF OESTRADIOL 17β HYDROXYSTEROID DEHYDROGENASE IN BREAST TISSUES: THE ROLE OF GROWTH FACTORS

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Summary—Oestradiol 17β-hydroxysteroid dehydrogenase (E_2DH) is present in normal and malignant breast tissues and regulates the interconversion of oestrone and the biologically active oestrogen, oestradiol. Studies we have previously carried out have indicated that concentrations of oestradiol and the conversion of oestrone to oestradiol are higher in breast tumours than in normal breast tissues. We are currently isolating and characterizing factors produced by breast tumours which are capable of stimulating E_2DH (reductive) activity. The production of such factors by breast tumours, which stimulate the conversion of oestrone to oestradiol, would provide a favourable oestrogenic environment to promote tumour growth and may account for the increased concentrations of oestradiol in breast tumours.

INTRODUCTION

Oestrogens have a central role in the development and growth of tumours of the breast with breast cancer remaining a major cause of death in Western women [1, 2]. The highest incidence of breast cancer occurs in postmenopausal women at a time when ovarian production of oestrogens has ceased. However, oestrogens continue to be formed in postmenopausal women. While there is some evidence to suggest that the adrenal cortex may produce a small amount of oestrogen [3], in postmenopausal women oestrogens are produced almost exclusively by the peripheral conversion of androstenedione to oestrone [4, 5]. Androstenedione, secreted mainly by the adrenal cortex, is converted to oestrone by the aromatase enzyme complex which is present in adipose and muscle tissues but also in normal and malignant breast tissues [6]. The contribution that local synthesis of oestrone from androstenedione makes to the total oestrogen content of breast tissues remains controversial [7, 8]. In a recent study which involved the use of an *in vivo* double isotope infusion technique, we found that in some, but not all, breast tumours, *in situ* formation of oestrone accounted for a major part of the oestrone found in breast tumours [9].

In breast tissues the activity of the aromatase enzyme complex is relatively low compared with that of oestrone sulphatase, the enzyme responsible for converting oestrone sulphate to oestrone [6, 7]. As plasma oestrone sulphate concentrations in blood of postmenopausal women are 8–10 times greater than unconjugated oestrone [10], it has been suggested that in breast tissues oestrone sulphate may be a more important source for the formation of oestrone than androstenedione [7, 11]. Oestrone sulphate is a polar steroid conjugate and little is known about the ability of oestrone sulphate to cross cell membranes and so be available for conversion to oestrone. To investigate this we have carried out a series of experiments in which either [^{3}H]oestrone sulphate or oestrone-[^{35}S]sulphate were infused into postmenopausal women with breast cancer [12, 13]. At operation samples of normal and malignant breast tissue, together with a blood sample, were obtained. By isolating the labelled oestrone sulphate from tissues and blood, it is possible to obtain a measure of the extent to which oestrone sulphate is taken up by breast tissues. After infusion of [^{3}H]oestrone sulphate a small but significant level of [^{3}H]oestrone sulphate was detectable in breast

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tissues. In contrast, no oestrone-[^{35}S]sulphate was detected after infusion of this labelled steroid conjugate. These results suggest that while it is possible that infused [^{3}H]oestrone sulphate is hydrolysed with the [^{3}H]oestrone being available for uptake and sulphation in breast tissues, oestrone sulphate, as such, does not appear to be taken up by breast tissues. It is of course likely that oestrone sulphate is hydrolysed in tissues other than the breast, such as the liver, and oestrone sulphate may therefore serve as a reservoir for the formation of oestrone. Hydrolysis of oestrone sulphate is from a slowly turning over pool and is likely to account for the continued presence of oestrone in plasma of postmenopausal women after treatment with the aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA). Peripheral conversion of androstenedione to oestrone is almost completely inhibited by 4-OHA, yet significant levels of oestrone are still detectable in blood [14, 15].

In addition to the aromatase and oestrone sulphatase enzyme complexes, the oestradiol 17β hydroxysteroid dehydrogenase (E_2DH) enzyme complex is also present in breast tissues. E_2DH mediates the interconversion of oestrone and the biologically active oestrogen, oestradiol. Two human placental E_2DH cDNA clones have now been isolated and used to examine the tissue distribution and regulation of E_2DH activity [16–18]. The cDNA encoding placental E_2DH identifies a 1.4 kb mRNA for this enzyme in tissues producing oestrogens with a 2.4 and 0.9 kb mRNA also being detected in other tissues [19].

The E_2DH enzyme complex is widely distributed in tissues throughout the body and our group and others have shown that this enzyme is present in both normal and malignant breast tissues [6, 20]. Thus, in addition to uptake from the circulation, it is also possible that *in situ* conversion of oestrone to oestradiol makes an important contribution to the concentration of oestradiol in breast tissues.

By use of an isotopic infusion technique it is possible to measure the peripheral conversion of oestrone to oestradiol and oestradiol to oestrone. Conversion of oestradiol to oestrone ($[\rho]^{E_2E_1}_{BB} = 20\%$) is about 4 times higher than the overall conversion of oestrone to oestradiol ($[\rho]^{E_1E_2}_{BB} = 5\%$), indicating that in most tissues of the body the oxidative function of this enzyme complex is predominant [21]. Conversion of oestradiol to the less potent oestrone is also the favoured direction of oestrogen metabolism in the endometrium. In this tissue E_2DH activity is progesterone-dependent [22]. The increase in E_2DH activity in the endometrium which results from the increase in progesterone production during the luteal phase of the menstrual cycle, decreases endometrial tissue concentration of oestradiol [23].

In breast tissues, however, it is not clear which direction of oestrogen metabolism (i.e. oxidative or reductive) is preferred. Measurements of mitotic frequency or the thymidine labelling index (TLI) in breast tissue biopsies have convincingly demonstrated that the peak of activity occurs on day 25 of the menstrual cycle [24]. This contrasts with the endometrium where the peak of mitotic activity is detected on day 14 of the menstrual cycle [25] and suggests that in the breast progesterone may stimulate cell proliferation. Using cultured human breast epithelial cells, Mauvais-Jarvis and his colleagues [26] have found that progesterone stimulates E_2DH (oxidative) activity. This has led these authors to claim that progesterone, as the endometrium, also has an "anti-oestrogenic" role in breast tissues, acting to increase the conversion of the biologically potent oestrogen, oestradiol, to the less active oestrogen, oestrone. Thus, two conflicting lines of evidence have emerged as to the role of progestogens in breast tissues and the effect of progestogens on E_2DH activity in breast tissues *in vivo* still awaits examination.

INVESTIGATION OF E_2DH ACTIVITY IN BREAST TISSUES

Our own studies, searching for evidence of some abnormality of oestrogen synthesis or metabolism in women with breast cancer, have extended over a number of years. Measurement of oestrone, oestradiol and oestrone sulphate concentrations and the free-oestradiol fraction in plasma obtained from normal women and women with breast cancer only revealed minor differences between women with or without breast cancer [27, 28]. Similarly, measurements of oestrogen production rates, metabolic clearance rates and rates of interconversion of oestrone and oestradiol revealed no major abnormality in women with breast cancer [21]. These studies did reveal for the first time, however, the possibility that differential regulation of the E_2DH enzyme complex might result in altered tissue exposure to oestradiol. A significant negative correlation was found between the conversion of oestradiol to oestrone ($[\rho]^{E_2E_1}_{BB}$)

and plasma dehydroepiandrosterone sulphate (DHA-S) concentrations. This finding suggested that some androgens may act to inhibit the inactivation of oestradiol, a concept which was confirmed for both endometrial [29] and breast tissues [30].

Subsequent measurements of breast tissue oestrogen levels revealed that the concentration of oestradiol was significantly higher in breast tumour than in normal breast tissue and that the concentration of oestradiol was increased compared with that of oestrone [31]. Increased concentrations of oestradiol in breast tumour tissue have now been confirmed by Van Landeghem *et al.* [32] and Vermeulen *et al.* [20]. In addition to measuring breast tissue oestrogen concentrations, we also measured E_2DH activity in the reductive (i.e. oestrone → oestradiol) and oxidative (oestradiol → oestrone) directions [31]. Surprisingly, in view of the increased oestradiol concentrations found in breast tumours, the oxidative activity of the E_2DH enzyme complex was significantly higher than reductive activity.

In an attempt to reconcile these conflicting results, we developed an isotopic infusion technique to enable oestrogen metabolism in breast tissue to be examined *in vivo* [33]. Results from this investigation revealed that in normal and malignant breast tissues of postmenopausal women little metabolism of oestradiol to oestrone occurred, a finding in keeping with a previous *in vitro* study [34]. In contrast, infusion of isotopically labelled [^{3}H]oestrone showed that significant conversion of oestrone to oestradiol occurred in breast tissues and that conversion of oestrone to oestradiol was significantly higher in breast tumours than in normal breast tissue. At the time that this study was carried out it was difficult to account for the conflicting data obtained from our *in vitro* and *in vivo* studies. However, it is now apparent that the E_2DH enzyme complex belongs to a superfamily of hydroxysteroid dehydrogenases (HSD) which includes 11β HSD. The 11β HSD enzyme complex also has oxidative and reductive functions. Results from recent investigations at the molecular level have revealed that while only one enzyme appears to be responsible for the oxidative and reductive functions of this enzyme complex, the reductive function is rapidly lost after expression of the protein [35]. Thus, it may only be possible to obtain an accurate measurement of E_2DH (reductive) activity by using an *in vivo* technique or alternatively by measuring reductive activity of this enzyme complex in cultured cells.

E_2DH ACTIVITY IN BREAST TUMOURS AND ADJACENT TISSUE

Our findings that in breast tumours conversion of oestrone to oestradiol and concentration of oestradiol were increased compared with normal breast tissue prompted us to explore the mechanisms by which this might be achieved. Such knowledge would have important clinical and therapeutic implications for the management of women with breast cancer.

Tumours of the breast most commonly occur in the upper outer quadrant of the breast and this observation initially led us to examine E_2DH activity in different breast quadrants.

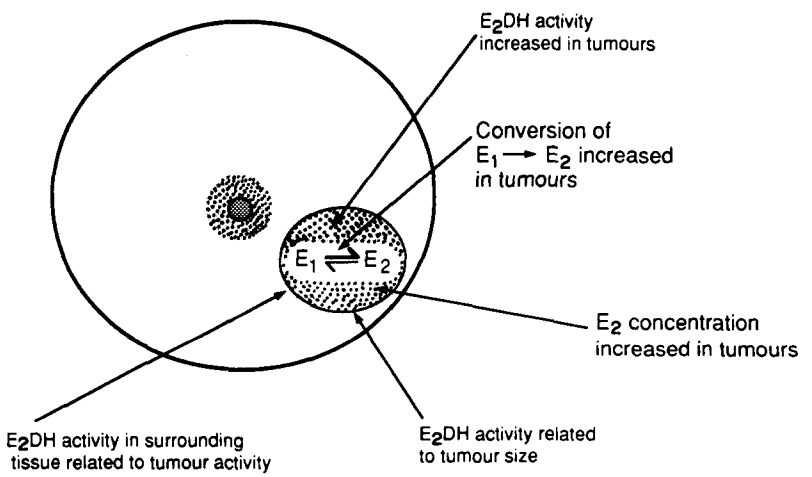

Fig. 1. Summary of results implicating tumour derived factors in the regulation of E_2DH activity in normal and malignant breast tissues.

E_2DH activity was found to be higher in the superior outer quadrants of breast tissue [36]. In the same study we also examined the relationship between E_2DH activity in tissue adjacent to breast tumours and tumour size. A significant correlation ($r = 0.75$, $P < 0.001$) between E_2DH activity in tissue adjacent to the tumour was found for malignant but not benign ($r = 0.28$, NS) tumours. O'Neill and Miller [37] have confirmed the association between tumour size and dehydrogenase activity in adjacent breast tissue. In view of the correlation between tumour size and E_2DH activity in adjacent tissue, we next examined whether there was an association between malignant tumour E_2DH activity and E_2DH activity in breast tissue some 2–5 cm from the tumour and again found a highly significant correlation [38].

Taken together the results from this series of *in vitro* and *in vivo* studies suggested the possibility that breast tumours produced factors which could influence: (a) the direction of oestrogen metabolism (i.e. in a reductive direction); and (b) the level of activity in tumours and adjacent breast tissues. Such a mechanism would provide a favourable oestrogenic environment to promote tumour growth. The findings which led us to postulate such a hypothesis are summarized in Fig. 1.

In a somewhat similar study, Miller and O'Neill [39] examined the relationship between tumour site and aromatase activity in different breast quadrants. Aromatase activity was always found to be highest in the quadrant of breast adipose tissue containing the tumour. Thus, it is possible that tumours also produce factors which enhance aromatase activity. An alternative explanation for this finding is that tumours develop in the breast quadrant producing most oestrone.

STIMULATION OF E_2DH (REDUCTIVE) ACTIVITY

To further examine the role that tumour derived factors may have in regulating E_2DH activity we initially employed a tissue explant culture system [40]. For this, finely minced breast adipose tissue was placed in a filter wick which was supported by a wire mesh in culture medium contained in a petri dish. The addition of tumour homogenates to the culture medium resulted in a significant stimulation of E_2DH activity.

We have subsequently shown that cytosol prepared from breast tumours [41], conditioned medium (CM) from cultured tumour fibroblasts [42] and breast cyst fluid (BCF) [43] all have the ability to stimulate E_2DH (reductive) activity in cultured MCF-7 breast cancer cells (Fig. 2). CM from malignant fibroblasts was also found to stimulate cell growth and E_2DH (reductive) activity to a greater extent than CM derived from benign or normal breast tissue [44]. Cytosol from malignant breast tumours stimulated E_2DH (reductive) activity in a dose-dependent manner, whereas cytosol from normal breast tissue was without effect. Cytosol from normal or malignant tissues had no effect on E_2DH (oxidative) activity. Results from this series of experiments strongly support the concept that factors present or produced by breast tumours and also in BCF are capable of stimulating E_2DH activity and enhancing the conversion of oestrone to oestradiol.

Further studies to examine the molecular weight(s) of factors present in tumour cytosol, CM or BCF have indicated that E_2DH (reductive) stimulatory activity is associated with proteins with molecular weights in the region of 30–70 kDa and it is possible that different stimulatory factors are present in the different

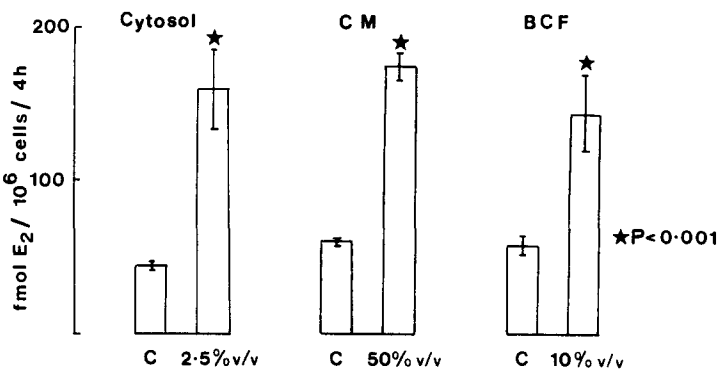

Fig. 2. Effect of breast tumour cytosol, CM from cultured tumour derived fibroblasts and BCF on E_2DH (reductive) activity in MCF-7 breast cancer cells.

fractions examined so far. While molecular weights in the region of 30–70 kDa are higher than those of most known growth factors, some are known to exist in higher molecular weight forms and some, such as mammary derived growth factor-1, have a molecular weight in this range [45]. Other factors have been previously isolated from the rat adrenal cortex [46] or human follicular fluid [47] and shown to be capable of stimulating steroidogenesis.

THE ROLE OF GROWTH FACTORS IN STIMULATING E_2DH (REDUCTIVE) ACTIVITY

Purification procedures now used to isolate the E_2DH (reductive) stimulatory factors include ammonium sulphate precipitation, ion exchange chromatography, HPLC gel filtration and polyacrylamide gel electrophoresis. To examine the possibility that stimulation of E_2DH (reductive) activity resulted from the presence of a known growth factor or protein, we have assayed the bioactive fractions obtained after chromatography for a number of different growth factors and proteins. Significant concentrations of a number of growth factors and proteins have been detected including IGF-I and IGF-II (concentration range 7–50 ng/mg protein). However, it was only possible to detect IGF-I and IGF-II after treating fractions with an acid–ethanol mixture to release these growth factors from their binding proteins. While IGF-like growth factors and/or their binding proteins are known to be secreted by a number of different cell lines, the role of binding proteins inhibiting or enhancing their biological activity remains controversial.

To further examine the potential role of insulin and insulin-like growth factors in stimulating E_2DH (reductive) activity, MCF-7 breast cancer cells were cultured in the absence or presence of IGF-I or IGF-II, at concentrations of 80 ng/ml. Both IGF-I and IGF-II significantly stimulated E_2DH (reductive) activity but had no effect on E_2DH (oxidative) activity [48]. Insulin also stimulated E_2DH (reductive) activity but only at supraphysiological concentrations (85–100 nM), suggesting that it may be acting to stimulate E_2DH (reductive) activity via cross-association with the IGF-I receptor.

ENDOCRINE CONTROL OF E_2DH ACTIVITY IN BREAST TISSUES

By examining the control of E_2DH activity in breast tissues, it is hoped that additional strategies will be developed for reducing oestradiol concentrations in breast tissues which will be of use in treating women with breast cancer. While the development of enzyme inhibitors is currently attracting considerable attention, no specific endocrine agents are yet available to inhibit E_2DH activity. In a recent study, we examined the effect of treatment with 4-OHA on aromatase activity and on breast tissue concentrations of oestrone. In a preliminary study we also examined the effect of such treatment on E_2DH activity. As shown in Fig. 3, E_2DH (reductive) activity in breast tumour tissue was significantly reduced after treatment with 4-OHA. In most studies reported so far, treatment with 4-OHA has resulted in a greater reduction in plasma oestradiol concentration than that for oestrone. Our results suggest that 4-OHA may also act to inhibit E_2DH activity, in addition to inhibiting the activity of the aromatase enzyme complex. It has also been reported recently that some of the newly developed "pure" anti-oestrogens may act in part to inhibit E_2DH activity [49]. In addition to inhibiting E_2DH (reductive) activity, it may also be possible to develop endocrine agents which selectively stimulate E_2DH (oxidative) activity and thus increase the rate of formation of the less potent oestrogen, oestrone, in breast tissues. In other studies we have examined the role of progestogens in regulating E_2DH activity in cultured breast cancer cells [50]. In contrast to the studies carried out by Mauvais-Jarvis and his colleagues [26] who only measured E_2DH (oxidative) activity, we have examined the ability of progestogens to moderate the oxidative and reductive activities of this enzyme complex. Results from our studies have shown that progesterone and synthetic progestins such as norethisterone and norgestrel, stimulate E_2DH (reductive)

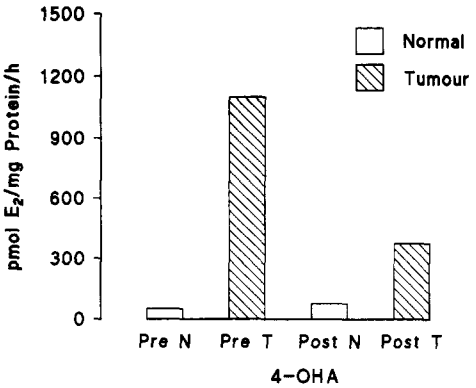

Fig. 3. Effect of 4-OHA on E_2DH (reductive) activity in normal (N) and malignant (T) breast tissues.

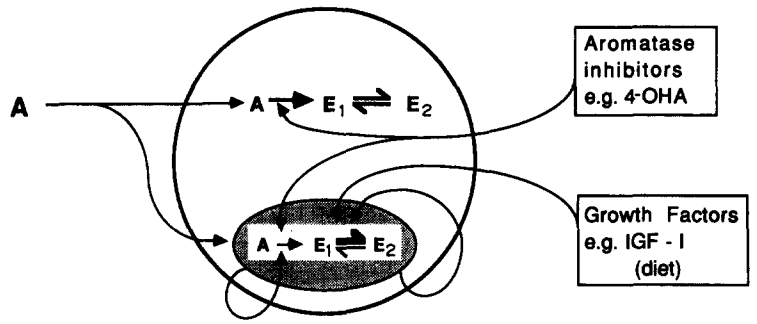

Fig. 4. Summary of factors modulating oestrogen synthesis in breast tissues. (A = androstenedione, E_1 = oestrone, E_2 = oestradiol, 4-OHA = 4-hydroxyandrostenedione, IGF-I = insulin like growth factor—type I.)

activity to a greater extent than E_2DH (oxidative) activity. It is possible, but remains to be confirmed, that in breast tissues progesterone stimulates E_2DH (reductive) activity during the second half of the menstrual cycle, at a time of decreasing blood oestrogen levels, and so enhance the *in situ* formation of oestradiol. Such a mechanism could reconcile the conflicting data reported by Anderson *et al.* [24] and Mauvais-Jarvis and colleagues [26] as to the role of progestogens in breast tissues.

In addition to the endocrine manipulation of E_2DH activity in breast tissues, investigations we have carried out have suggested a mechanism by which diet might influence oestrogen metabolism in breast tissues. As already noted, our *in vitro* studies have shown that IGF-I can stimulate E_2DH (reductive) activity in MCF-7 breast cancer cells. We have recently shown that consumption of a calorie-restricted diet for a 2-week period results in a significant (60%) decrease in blood IGF-I concentration [51]. Thus, it is possible that the type of diet and/or number of calories consumed may influence E_2DH activity in tissues such as the breast. Such a mechanism may help to explain the difference in the incidence of breast cancer found in Eastern and Western countries.

CONCLUSIONS

In this review we have summarized the evidence we have obtained showing that E_2DH activity has an important role in regulating concentrations of oestradiol in breast tumours. A summary of our major findings is shown in Fig. 4. While we have obtained a considerable amount of evidence to support the concept that breast tumours do produce factors which are capable of modulating E_2DH activity, the nature and mechanisms by which such factors act requires further investigation. The major problems that remain to be resolved include whether more than one enzyme is involved in the interconversion of oestrone and oestradiol and what determines the direction of oestrogen metabolism in different tissues. The molecular techniques that are now available should enable us to obtain answers to these vital questions.

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