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GENERAL REVIEW

ENDOCRINE TREATMENT FOR BREAST CANCERS: BIOLOGICAL RATIONALE AND CURRENT PROGRESS

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Summary—Endocrine therapy is a major treatment modality for the systemic management of breast cancer. In comparison with alternatives such as chemotherapy, hormone manipulations have the advantage of lower toxicity but suffer from the disadvantages of producing responses in only 30–40% of patients with metastatic disease and seldom being curative. Nevertheless in recent years there have been significant advances in the endocrine treatment of breast cancer which have stemmed from a better understanding of the sources from which breast tumours may be supplied with hormones, the mechanism by which hormones regulate tumour proliferation and the more accurate identification of hormone sensitive tumours. As a result agents such as antioestrogens, aromatase inhibitors, LHRH agonists have largely superseded surgical and radiological ablation of endocrine organs. The major reduction in morbidity associated with these medical regimes means that they are much more acceptable to patients and may be used as adjuvants to local treatment of the breast in patients with "earlier" stages of the disease. At the same time patients can now be offered rational treatment selected on the basis of tumour biology rather than on more empirical criteria. The aims of this review are to provide details of the research which has led to this progress in endocrine treatment of breast cancer and to put into perspective the prospects for further advances.

HISTORICAL REVIEW

The first indication that the growth of breast cancer may be under endocrine control was remarkable in that it was put forward over 100 years ago without the comprehension of the nature of hormones. Thus in 1889, a German surgeon, Schinzenier noted that atrophy of the breast normally followed cessation of ovarian function and suggested that ovariectomy might lead to regression of breast cancer [1]. However, he never performed the procedure and it was left to Sir George Beatson to describe beneficial effects of surgical castration in premenopausal women with breast cancer [2]. It is now evident that ovariectomy, whether by surgical or radiological means, will produce beneficial effects in about one third of premenopausal patients with advanced breast cancer [3]. With the discovery and manufacture of synthetic glucocorticoids such as cortisone, which can be given as replacement corticosteroid therapy, procedures such as adrenalectomy and hypophysectomy became practical propositions. Endocrine deprivation therapy could then be performed in postmenopausal women. Subsequently it became clear that ablation of either the adrenals or pituitary could produce regression rates of 30–40% in postmenopausal patients with advanced breast cancer [4, 5]. Conversely and paradoxically, pharmacological doses of a variety of steroid hormones, including androgens [6], oestrogens [7], progestogens [8] and corticosteroids [9] are capable of eliciting meaningful responses in a similar percentage of patients with advanced breast cancer.

This brief historical review illustrates the dilemma associated with endocrine therapy for breast cancer, viz. it is possible to produce objective remissions but only in the minority of patients. Furthermore, ultimately most women still die of cancer but with evidently hormone-unresponsive disease. If, therefore, hormone manipulations are not curative, their acceptability for treatment will depend largely on their relative lack of toxicity in comparison with other major therapeutic modalities such as chemotherapy. There has thus been a drive to develop reversible and less toxic forms of hormone treatment. Agents such as LHRH

agonists, antioestrogens and aromatase inhibitors, coupled with the ability to identify tumours most likely to respond to such regimes represent major steps forward. This progress has largely stemmed from a basic understanding of the mechanisms by which hormones are supplied to breast cancers and exert their action within target cells. Similarly, with fundamental knowledge, it is possible to illuminate the processes which occur during the transition from hormone dependence to autonomy and suggest strategies for either retarding this transformation or striking against tumour cells irrespective of their hormone sensitivity. These are the subject areas of this review which seeks to illustrate how current research into the biology of the breast and its tumours and the biochemistry of antihormone drugs are leading to a more rational management of breast cancer and offering hope for the future.

SENSITIVITY TO INDIVIDUAL STEROIDS

Cell lines derived from breast cancers may show sensitivity in culture to a variety of hormones including oestrogens [10], androgens [11], progestogens [12, 13] and corticosteroids [13]. Primary breast cancers may also possess high affinity receptor sites which specifically bind these same steroids [14–17]. Tumour growth may therefore be potentially dependent upon one or more of these steroids and it is often difficult to attribute responses to specific hormones when endocrine therapies may affect the level and action of many hormones.

However, the most persuasive case for the involvement of a single steroid class surrounds oestrogens. Thus all major hormonally-based therapies for breast cancer have in common the capacity either to reduce circulating oestrogens or antagonise the effect of oestrogen (Table 1). Furthermore the single best predictor for response to hormone therapy, irrespective of its type, is the presence of oestrogen receptors within tumours [18]. The anecdotal reports of accelerated tumour growth following administration of oestrogen to patients with breast cancer would also be compatible with the key involvement of oestrogen [9, 19, 20]. While not wishing to ignore the potential role of other hormones either in their own right or as modifiers of oestrogen action, the following discourse will be largely concerned with oestrogens.

Table 1. Major endocrine therapies—effects on oestrogen biosynaethesis/action

Therapy	Potential mechanism of action
Ovariectomy	Ablation of major source of oestrogen in premenopausal women
Adrenalectomy	Eliminates major source of androgen precursor of oestrogen in postmenopausal women
Hypophysectomy	Removes pituitary hormones trophic to ovarian and adrenal biosynthesis of oestrogenic hormones
LHRH-agonists	Down-regulate LHRH drive for ovarian production of oestrogens
Aromatase inhibitors	Prevent biosynthesis of oestrogens from androgens
Antioestrogens	Block action of oestrogen at its receptor
Pharmacological doses of steroids (e.g. diethyl stiboestrol)	Down regulate oestrogen receptors

MECHANISMS OF HORMONE ACTION

Whilst steroids can indirectly affect the growth of breast cancer by influencing polypeptide hormones such as "oestromedins" secreted by the pituitary and other endocrine organs [21], the major actions of steroids are probably direct on tumour cells. Direct effects are largely mediated through specific intracellular receptors which interact with the genome to programme hormonal response [22, 23]. (Antihormones such as tamoxifen are thought to have their major action by competing with natural ligands for their receptors [24].) Whether the response elicited by receptor–genome interaction directly modifies cellular proliferation or whether growth effects are mediated via secondary response modifiers is unclear. However, in cell lines of breast cancer, oestrogens may (i) induce the secretion of mitogenic factors [25, 26] and (ii) stimulate the production of proteins such as cathepsin D [27] and plasminogen activator [28] which may degrade the extracellular matrix and thereby accelerate tumour invasion and metastasis. These observations have important implications. For example, if the actions of steroid hormones are mediated by growth factors the tropic effect of steroid hormones should be mimicked by growth factors. Conversely anti-growth factor strategies should cause regression of steroid hormone-dependent tumours.

Experimental systems do not completely support these concepts. Thus, whilst oestrogen may stimulate the secretion of TGFα and IGF-I by hormone-dependent breast cancer cell lines [25, 26], these individual growth factors, even if over expressed, are incapable of replacing oestrogen as an essential requirement for tumour growth in immunosuppressed animals [29]. Furthermore, whilst a cocktail of

growth factors, as may be present in media conditioned by *in vitro* growth of tumour cells may support oestrogen-dependent growth, the resulting tumours fail to reach the size as would be produced by oestrogen [30]. Thus, the full growth-promoting effects of oestrogen seem to require other agents in addition to secreted growth factors. The sequalae from the observations are that therapies directed against oestrogen-induced growth factors are unlikely to be as efficient in causing tumour regression as antioestrogens targetted against oestrogen receptor protein, and conversely that successful antioestrogen therapy is not simply mediated via antagonism of growth factor production but embodies a fuller panoply of actions.

PROGRESSION TO HORMONE DEPENDENCE

Clinical experience shows that even following successful endocrine treatment, most hormone-dependent tumours resume growth and will subsequently kill their host [31]. It is thus important to determine how the process of autonomy develops. There are several possible mechanisms. First the endocrine system may adapt to constraints put on it and compensate by producing more hormone, perhaps from an alternative source. Secondly, breast cancers like other malignancies display an unstable phenotype [32] and, perhaps at an early stage, clones of hormone-independent cells develop which following hormone deprivation will selectively emerge from hormone sensitive clones. Lastly it is possible that during treatment hormone-dependent cells learn to bypass the requirement for an external source of hormone. It is worth considering each of these possibilities in a little more detail.

Increased levels of circulating steroids have been reported at the time of relapse after successful endocrine treatment [33]. Whilst this would be consistent with adaptation of the endocrine system resulting in renewed tumour growth, it could also be the consequence rather than the cause of progressive disease (for example progressive metastatic disease might reduce metabolic clearance of hormones). It also has to be emphasised that in most patients treatment failure is not associated with a change in the circulating steroids [34].

There is evidence that progression to hormone dependence is caused by the outgrowth of hormone-independent clones of cells which have been present from initiation of therapy. Thus cellular heterogeneity within tumours with regard to hormone sensitivity and hormone receptors is well documented [35, 36]. Selective cell-kill can also be demonstrated after successful hormone therapy [37, 38]. Furthermore second responses to further endocrine manipulations are also more likely in tumours responding to first line therapy [39]. This would be compatible with successive destruction of cellular populations with differing hormone sensitivity. Conversely however, endocrine therapy does not always produce the expected phenotypic change. For example, if oestrogen receptors status is a marker of hormone dependence and tumour regrowth following successful treatment is caused by resistant oestrogen receptor-negative cells outgrowing from compromised oestrogen receptor-positive clones, then the resulting hormone-independent tumour should be oestrogen receptor-poor or negative. In practice this is not the case, the resulting tumour being most often still oestrogen receptor-positive [40]. Results from model systems are consistent with clinical experience, oestrogen receptor-positive cell lines of breast cancers adapting to grow in culture in the absence of oestrogen while still maintaining their receptor status [41].

In order to understand the acquisition of autonomy, more fundamental knowledge is needed of the cellular processes involved. Steroid deprivation appears to accelerate a phenotypic or epigenetic adaption which involves a stable alteration in gene expression in at least some tumour cell populations. Thus long-term growth of breast cancer cell lines in culture without steroids results in an ordered, reproducible series of phenotypic changes culminating in loss of both cellular and molecular steroid-sensitive parameters [42]. Initial changes are reversible on addition of steroids but later changes are irreversible. Interestingly loss of sensitivity to one steroid may be accompanied by loss of response to other steroids and altered response to serum but without loss of oestrogen receptors or certain steroid inducible molecular markers [43, 44]. But what are specific phenotypic changes that lead to autonomy? Three possibilities are considered.

Firstly, certain breast cancers have the ability to synthesize oestrogen [45] (see later) and acquisition of such steroidogenic potential may allow tumours to become independent of external sources of hormones. However, there is little evidence that progression to autonomy is associated with enhanced capacity for steroid

biosynthesis or that hormone-independent cancers are more likely to synthesize oestrogen than hormone-dependent tumours.

Secondly, because oestrogens can stimulate the secretion of mitogenic factors by tumour cells hormone independence may result from constitutive production of growth factors which normally require to be hormonally induced. In support of this a tendency has been reported for hormone-independent cancer cell lines to secrete higher amounts of growth factors than hormone-dependent cell lines [46]. Furthermore transfection of v-Hras genes into hormone sensitive cells can cause transformation into evidently hormone-independent phenotype, this being associated with elevated secretion of growth factors by the transformed cells [47]. However this effect may be coincidental and, as indicated earlier, transfection studies in which cells have been engineered to over produce growth factors have not shown that this leads to a hormone-independent phenotype. Preliminary results on growth factor levels in primary breast cancers [48] also do not support the concept that hormone-independent tumours are autonomous on account of enhanced growth factor synthesis.

Lastly oestrogens are only one of many agents that can influence tumour growth and endocrine unresponsive growth may result from a switch to dependence on other mitogens which are unrelated to steroids or steroid inducible factors.

PREDICTION OF HORMONE RESPONSIVENESS

A major advance in the management of breast cancer has been the development of the oestrogen receptor (ER) assay to predict tumour hormone responsiveness. Between 50 and 60% of patients with ER-positive tumours respond to endocrine therapy. In contrast, less than 10% of patients with ER-negative tumours benefit from hormone manipulation. ER assays are now used routinely to select patients for endocrine therapies. However, a problem still remains in that about half of patients with ER-positive tumours will not respond to hormonal manipulations. Further discriminators are therefore required.

One approach in the search for markers of hormone sensitivity has been to explore measurements of proteins or mRNA induced by oestrogen, these being potential indicators of functional oestrogen response mechanisms. Foremost amongst these parameters is the progesterone receptor (PgR) whose production in breast cancers seems to be under the control of oestrogen [49]. In combination ER and PgR can provide powerful discrimination for endocrine responsiveness of breast cancer; tumours possessing both receptors respond well to endocrine therapy, while those lacking both receptor rarely respond and tumours with ER but without PgR have intermediate response rates [49, 50]. It is also possible that tumour hormone sensitivity reflects the balance between mechanisms mediating steroid hormones and those antagonizing their action. In this respect, level of cyclic AMP binding proteins may be a marker of oestrogen antagonism. Expression of oestrogen receptors in a ratio with cyclic AMP binding proteins may significantly increase the discrimination between hormone-dependent and -independent tumours beyond that achieved by oestrogen receptors alone [51]. Accurate identification of hormone-responsive tumours on an individual basis is becoming a realistic prospect.

SOURCES OF TUMOUR OESTROGENS

The ovary represents the principle source of oestrogen for breast cancers in premenopausal women and this is reflected in the regression of hormone-dependent cancer following castration of premenopausal patients. After the menopause, however, the ovary produces minimal amounts of oestrogen [52] and the adrenal cortex also secretes negligible quantities [53] although both organs are responsible for large quantities of androgens [52–54]. These androgens may be used as the immediate substrate for oestrogen by the aromatase enzyme in a variety of peripheral tissues, including fat [55], skin [56] and certain breast cancers [45, 57]. Such peripheral oestrogen biosynthesis, particularly that in adipose tissue, may be physiologically important in postmenopausal women. For example plasma levels of oestrogen are directly related to body weight [58] and degree of obesity [59] in postmenopausal subjects suggesting that oestrogen biosynthesis in adipose tissue is primarily responsible for concentrations of circulating oestrogen in these women. Controversy exists as to whether aromatase in distant adipose tissue or that locally within breast adipose tissue or breast cancers is the immediate source of tumour oestrogen but some form of peripheral tissue is responsible. This has important implications for treatment strategies. Most peripheral tissues are not amenable to either surgical or

radiotherapeutic ablation and therefore some form of chemotherapeutic intervention is necessary. Hence the interest in drugs which specifically inhibit the aromatase enzyme. The factors which control aromatase activity in peripheral tissues are largely unknown but they appear to be different to those controlling oestrogen biosynthesis in classical endocrine organs. Thus gonadotrophin do not appear to regulate aromatase in any peripheral tissue [60]. In contrast a number of agents such as glucocorticoids, cyclic AMP and phorbol esters are capable of inducing aromatase in human adipose tissue [60, 61]. Conversely several growth factors and cytokines such as EGF, TGFα, TNF and IL1β are inhibitory [62]. Since, as discussed earlier, many of these polypeptide factors are produced by breast cancers the potential for paracrine regulation and interaction between tumour and adipose tissue within the breast exists [63]. This type of phenomenon might explain the observation that aromatase activity in mammary fat is elevated in areas of the breast in which cancer is located [64].

ENDOGENOUS OESTROGENS WITHIN THE BREAST

Concentrations and patterns of oestrogen differ markedly between the circulation and breast tissues, particularly in postmenopausal women [65, 66]. Amongst the most striking disparities are the observations that (i) tissue concentrations of oestrogen are similar in pre- and postmenopausal women despite the marked fall in peripheral plasma levels after the menopause, (ii) in postmenopausal women levels of oestrogen are significantly higher in the breast compared with the circulation, (iii) while oestrone and its sulphate predominate over oestradiol in the circulation, levels of oestradiol are similar to or higher than oestrone in breast tissue.

In general these observations apply to both cancerous and non-malignant components of the breast but there is a trend for differences to be more marked in malignant tumours. Thus oestradiol levels tend to be higher in cancers compared with normal or benign breast tissue [65]. Breast adipose tissue also has levels of oestrogen markedly in excess of those in peripheral plasma but these consist mainly of oestrone [67]. These considerations indicate that endogenous concentrations of oestrogen within the breast do not necessarily reflect those in the circulation.

Two main causes could be responsible for these distinctive profiles of oestrogen within mammary tissues: selective uptake of specific oestrogens from the circulation against a concentration gradient or active synthesis and metabolism within the breast. Perfusion studies [68, 69] show that the breast can take up and concentrate both androgens and oestrogens and that the accumulation can be selective and vary between breast cancer and normal breast. For example in breast cancers the uptake of oestradiol seems to exceed that of oestrone.

The mechanism by which this selective uptake occurs is unknown, but it is not unreasonable to postulate the presence of intracellular high affinity binding proteins capable of maintaining tissue levels of oestrogen. However, no binding protein has been identified whose quantitative presence correlates with tumour concentrations of oestrogen. Thus, while oestradiol levels tend to be higher in oestrogen receptor-positive tumours, no correlation exists between levels of receptor and oestradiol [65, 70]. Levels of both oestrone and oestradiol in receptor-negative tumours are also markedly in excess of those in plasma. Involvement of proteins with lower affinity and higher capacity for oestrogen cannot be excluded.

Both *in vivo* and *in vitro* studies show that breast tissues have the capacity for oestrogen biosynthesis and interconversions [45, 65, 71, 72]. Local activity of two transformations could potentially account for endogenous tumour levels of oestrogens, namely the aromatization of androgens to oestrogens and the interconversion of oestrone to oestradiol. However, although these activities have been shown to be present in breast tissues [72–74] and influenced by local factors [63, 74], no correlation has been reported between either aromatase or 17β-hydroxysteroid dehydrogenase and tumour levels of oestrogens [46, 75, 76]. As the extent of oestrogen production and uptake is highly variable between tumours [69], it may be that local metabolism is primarily responsible for endogenous oestrogens in some cancers whereas selective uptake is the major mechanism in others.

STEROID METABOLISM WITHIN THE BREAST

Whilst steroid metabolism within the breast may not correlate with levels of endogenous steroids, positive relationships have been reported between several aspects of metabolism

and the presence, stage and hormone dependence of breast cancer [76]. Thus enhanced levels of aromatase activity in breast adipose tissues are associated with the presence of breast cancer [67] and those of 17β-hydroxysteroid dehydrogenase with stage of disease [76, 77]. Tumour oestrogen biosynthesis and sulphurylation have also been reported to be associated with response to aminoglutethimide [78] and adrenalectomy [79] respectively in patients with advanced breast cancer.

Steroid metabolism in the breast may also be influenced by autocrine, paracrine and endocrine factors [80]. Since pathways of hormone activation and deactivation exists, the potential of certain systemic endocrine therapies to modify local metabolism within the breast may yet be proved to be an important feature in their mechanism of action.

NOVEL FORMS OF ENDOCRINE THERAPY

It is important that the greater understanding of tumour biology and its hormonal control is eventually translated into improved forms of endocrine therapy. The next section will consider specific areas in which this objective is being achieved.

LHRH AGONIST ANALOGUES

Gonadal production of steroid hormones is under the control of pituitary gonadotrophins whose secretion is in turn regulated by the hypothalamic releasing factor, LHRH [81]. In recent years analogues of LHRH have been synthesized which have agonist properties [82, 83]. When administered acutely LHRH agonists cause a rapid release of gonadotrophins into the circulation [83, 84] but when given chronically the analogues produce paradoxical effects in that plasma gonadotrophins fall [83–85]. In premenopausal women, this results in a decreased drive to the ovaries and circulating levels of oestrogens fall to castrate values [83–86]. These endocrine effects gave rise to the concept of using LHRH analogues as a form of medical ovariectomy in premenopausal women with advanced breast cancer. The results of several studies using different types and regimes of LHRH agonist have confirmed the validity of such an approach and demonstrated a therapeutic benefit similar to that produced by either radiation-induced or surgical castration [82, 85, 87–89].

The immediate advantage of LHRH agonists is that unlike other forms of castration, treatment is reversible, ovarian function returning on discontinuation of LHRH agonist administration [83]. The relative lack of side effects and easy administration of newer preparations also contrast with the trauma and morbidity of surgical castration. These are important considerations if the majority of patients fail to respond to endocrine measures.

Whilst the major clinical studies of LHRH agonists have been logically performed in premenopausal women, small numbers of postmenopausal women have also been treated with the preparations. Interestingly about 10% of such patients with advanced breast cancer gained meaningful responses [89–91]. The mechanism by which these benefits have been achieved is not immediately obvious. Effects on circulating oestrogen are relatively minimal [91, 92] although more substantial decreases in androgen levels may be observed [93]. It may be more relevant that direct inhibitory effects of LHRH and its analogues have been reported in breast cancer cells in culture [94, 95] and that certain breast tumours appear to have specific binding sites for LHRH (agonists) [96].

ANTIOESTROGENS

The use of non-steroidal antioestrogens, particularly tamoxifen, has been a major advance in the treatment of breast cancer. Since tamoxifen's first use in 1971 as a palliative regime in advanced breast cancer, results from almost 4000 women have shown that about one third of patients obtain a complete or partial response [97]. Because of the drug's efficacy and general lack of toxicity, tamoxifen is now established as first-line endocrine therapy in postmenopausal patients. Its use also now extends into the adjuvant setting in which systemic therapy is given to delay or prevent the progression of occult metastatic disease. Recently mega-analyses of a large number of randomized trials has shown that adjuvant tamoxifen not only retards the appearance of recurrent disease but significantly reduces mortality in women aged 50 yr or older [98]. There are, however, still a number of issues which remain to be clarified and for which laboratory investigations may provide some guidance. These include (i) optimization of the length of time for which adjuvant tamoxifen requires to be given, (ii) the potential

benefits of tamoxifen in oestrogen receptor-negative tumours and (iii) the future of more potent and specific antioestrogens.

The optimal time for which tamoxifen requires to be given in an adjuvant setting is largely dependent upon the mechanism of the drug's action. If tamoxifen is tumouricidal, it need only be given for the period necessary for maximum cell-kill; if tumouristatic, the drug potentially requires to be taken indefinitely. Most evidence points to tamoxifen being a cytostatic agent. For example, in athymic mice bearing hormone-sensitive human breast cancer cells, tamoxifen will retard tumour development and, as long as the drug is administered, tumours fail to appear. However, once tamoxifen therapy is withdrawn, providing oestrogen is present, the animals invariably develop tumours [99]. This suggests that tamoxifen is unable to destroy cancer cells and disease can be reactivated under the appropriate stimulus. Consistent with this is the observation that the addition of tamoxifen to cultures of hormone-sensitive human breast cancer cells is associated with accumulation of cells in the G_0/G_1 stage of the cell cycle [100]. Definitive data from clinical trials in which different durations of therapy are directly compared is not yet available but the signs are that more prolonged exposure to tamoxifen will offer further benefits [101, 102].

Most agree that the major benefits of tamoxifen stem from interaction with the oestrogen receptor. Thus the likelihood of response to tamoxifen in postmenopausal patients with advanced breast cancer is much higher in ER-positive tumours compared with ER-negative tumours [97]. Nevertheless, a small but consistent proportion (10%) of ER-negative tumours do respond to tamoxifen. Furthermore, whilst most trials of adjuvant tamoxifen suggest the drug is more effective in patients with ER-rich cancers [103], major studies suggest that benefits spread over the whole of ER values including tumours which are ER-negative [103, 104]. These results imply that tamoxifen may be active other than by a pure antagonism of the oestrogen receptor. Interest has been reawakened in alternative mechanisms of action. It has been shown that growth-inhibitory concentrations of tamoxifen can markedly induce TGFβ in oestrogen receptor-positive breast cancer cells. This is then capable of inhibiting the growth of co-cultured ER-negative tumour cells [105]. Tamoxifen may also induce TGFβ production by stromal fibroblasts [104]. This observation has led to the proposal that tamoxifen-induced secretion of TGFβ and other negatively regulatory growth factors by non-malignant cellular elements may inhibit the growth of ER-negative tumour cells by a form of paracrine communication [104]. A similar scenario can be developed for interaction between ER-positive and ER-negative cells if tamoxifen either inhibits the oestrogen-induced secretion of growth promoting factors or stimulates the production of inhibitory factors by ER-positive tumour cells, such diffusable factors influencing the behaviour of adjacent ER-negative cells.

The reason for seeking novel antioestrogens is based on the recognition that so far all antioestrogens used clinically have partial agonist or oestrogenic activity [106]. As a consequence, antioestrogens such as tamoxifen are unable to block completely the trophic actions of oestrogens [107]. The development of an antioestrogen devoid of intrinsic oestrogen activity and which also completely blocks the trophic action of oestradiol might have considerable potential and perhaps increase the numbers of complete tumour remissions. It is thus interesting that Wakeling and Bowler [108] have recently shown that 7α-alkylamide analogues of oestradiol have the pharmacological characteristics of a pure antioestrogen. One of these compounds (ICI164,384) appears completely free of oestrogenic activity in rodents [108, 109] and blocks the trophic actions of exogenous and endogenous oestradiol and of partial agonist antioestrogens like tamoxifen [108, 110]. ICI 164,384 is also peripherally selective, producing complete involution of the uterus without affecting LH secretion in intact female rats. In oestrogen-responsive human breast cancer cells, the pure antioestrogen is a more potent and effective inhibitor of cell growth than tamoxifen [111]. This implies that a pure antioestrogen may provide a more effective therapy for breast cancer. However, a word of caution needs to be sounded in that some of the oestrogenic effects of partial oestrogen agonists may be beneficial —for example, effects on bone loss and blood biochemistry. It is just possible that in tamoxifen we already have an ideal agent which has sufficient antioestrogenic potential to inhibit cancer growth effectively but by virtue of its oestrogenic properties can protect against bone loss and other symptoms associated with oestrogen deprivation.

AROMATASE INHIBITORS

The concept of inhibiting oestrogen biosynthesis with drugs is attractive. In comparison with endocrine ablative surgery, this approach has several advantages in that (i) the action of aromatase inhibitors are self-limiting and if therapy proves ineffective, oestrogen levels should return to normal on discontinuation of treatment, (ii) specific aromatase inhibition will primarily affect oestrogens alone and minimize side-effects and morbidity not associated with oestrogen deprivation, (iii) oestrogen levels will be reduced irrespective of site of biosynthesis—as a result aromatase inhibitors are capable of suppressing oestrogen levels beyond those achievable by surgical ablation of endocrine glands.

Oestrogens are the end-points of a sequence of steroid transformations. Blockage of any of these conversions potentially leads to decreased oestrogen production but more specific suppression will theoretically result from inhibition of the final step in the pathway which is unique to oestrogen biosynthesis, the aromatase reaction. The key role of aromatase in oestrogen biosynthesis has generated considerable interest in putative inhibitors of the enzyme.

Aminoglutethimide is to date the only drug with antiaromatase activity which has been used extensively in clinical trials. The drug was originally introduced for treating metastatic breast cancer as a form of medical adrenalectomy [112]. Doses were given which inhibited adrenal cholesterol side cleavage and as a result secretion of corticosteroids was markedly reduced and replacement corticosteroid was required. Subsequently it was shown that aminoglutethimide-hydrocortisone also blocks peripheral conversion of androgens to oestrogens *in vivo* [113] and this seems to be the primary cause of the regime's antitumour effects (which in postmenopausal women are similar to those reported for other endocrine therapies in terms of response rate, duration of remission and site of response) [114]. As an aromatase inhibitor, however, aminoglutethimide has several drawbacks in that (i) it is not particularly potent, (ii) it lacks specificity and has side-effects not associated with its antiaromatase inhibition and (iii) it requires the concomitant administration of corticoids. There has therefore been an interest in developing newer aromatase inhibitors without these disadvantages. The aromatization of androgens to oestrogens involves three hydroxylations, each using NADPH as an electron donor and a prosthetic cytochrome *P*450 for electron transfer. Aromatase inhibitors have therefore been divided into two types—type I agents which combine with the catalytic site of the enzyme (and are invariably substrate analogues) and type II inhibitors which interact with the cytochrome *P*450 moeity.

Type II inhibitors, of which aminoglutethimide is an example, often suffer from lack of specificity in that other hydroxylases also have cytochrome *P*450 prosthetic groups and may be inhibited. However, drugs may have differing affinity towards cytochrome *P*450 in different enzymes and in general cytochrome *P*450 in aromatase is more susceptible than other steroid hydroxylases to aminoglutethimide-like drugs [115]. These properties are exploited in an imadazole derivative of aminoglutethimide (CGS 16949A) which is about 1000-fold more potent as an aromatase inhibitor *in vitro* than aminoglutethimide [116] but at concentrations which maximally inhibit aromatase, CGS 16949A has minimal effects on other cytochrome *P*450 containing enzymes [117]. This means the drug may be administered to patients without the need for corticoid replacement. Preliminary results in treating postmenopausal women with advanced breast cancer have shown promising results with few side effects [118].

Type I inhibitors as substrate analogues tend to be more specific than type II inhibitors. A subset of substrate analogues known as suicide inhibitors are particularly interesting. These compounds have little inhibitory activity *per se* but are metabolised by the catalytic site of aromatase to reactive intermediates which bind covalently and irreversibly to the enzyme causing loss of activity. The enzyme is thus inactivated as a consequence of its own function. Suicide inhibitors would be expected to be extremely specific since they should only inactivate those enzymes for which they are substrate. Prolonged effects can also be predicted *in vivo* as the enzyme is not only inhibited but inactivated even after free inhibitor is no longer present; resumption of oestrogen production depends on the synthesis of new aromatase molecules. Such an inhibitor is 4-hydroxyandrostenedione which produces a time-dependent inactivation of the aromatase enzyme and is about 40-fold more potent than aminoglutethimide [119]. 4-Hydroxyandrostenedione has been used to treat advanced breast cancer in postmenopausal

women. The drug produces tumour remissions in about 33% of patients and disease stabilization in a further 15% [18, 120]. The particular advantages of 4-hydroxyandrostenedione are low toxicity and the lack of need for corticoid replacement.

The development of novel specific potent aromatase inhibitors therefore offers the promise of reduced toxicity and enhanced efficacy over traditional drugs. However, some perspective must be maintained especially in terms of the search for even more potent inhibitors. Thus whilst current aromatase inhibitors already inhibit peripheral aromatase almost completely [113] levels of circulating oestrogen fall by only 40–85% [121]. Exogenous oestrogens (such as in the diet) and other steroids with oestrogenic activity such as androstenediol are not affected by aromatase inhibitors and may maintain hormone-dependent growth [122].

Aromatase inhibitors are also less effective in premenopausal women [123]—even if sufficiently large doses are given to block the higher levels of aromatase in the ovary, compensatory reflex feed-back loops cause gonadotrophins to rise and result in secondary increases in both androgen substrate and aromatase in the ovary. Reliable oestrogen suppression in premenopausal women by aromatase inhibitors will therefore additionally need measures by which to interfere with feed-back controls.

NOVEL METHODS OF OESTROGEN DEPRIVATION

These, by their nature, are speculative. For example, an alternative to blocking oestrogen biosynthesis by inhibiting the aromatase system would be to devise inhibitors of the C17–20 lyase enzyme. As this enzyme is higher in the sequence of reactions leading to oestrogens it would theoretically reduce both androgen and oestrogen biosynthesis. This may have potential advantages as certain androgens, particularly those of the 5-ene series, are capable of eliciting oestrogenic responses [126]. The prototype drug is Ketoconazole which inhibits C17–20 lyase but because its mechanism of action is via cytochrome *P*450 hydroxylase, at high doses it also blocks cortisol biosynthesis [124]. The development of more specific drugs should lead to inhibition of sex steroids alone.

It is also possible that specific antioestrogen effects may be achieved using an immunological or molecular biological approach. Antibodies against oestrogen and its receptor are available and have therapeutic potential. Greater efficacy could be achieved by coupling the antibodies to cytotoxic agents or radioisotopes. There are however immediate problems to overcome, such as the specificity of targets, the entry of antibodies into cells (oestrogen receptors being located in the nuclei [125]) and the uncoupling of the antibodies at non-target sites. Anti-sense mRNA against oestrogen receptor or its induced products may also potentially block oestrogen action. However, little is known about the dose–response requirements, entry of mRNA into tissue and the specificity of such approaches.

Insofar as many of the products of oestrogen interaction with its receptor have biological activity and might give the tumour growth advantage and aid its metastatic spread, there is great interest in developing analogues, antibodies or antisense mRNA against such targets. Foremost amongst these targets are oestrogen-induced growth factors. This is an exciting approach, but some caution is needed. It seems unlikely that the proliferative response to oestrogen is due to a single growth factor or that the cocktail of factors will be similar in all oestrogen-sensitive tumours. Furthermore as most of the factors induced by oestrogen appear identical to those required by normal tissue, such approaches, in common with most other chemotherapeutic regimes will be associated with side-effects, particularly in tissues containing a high proportion of dividing cells. The hope must be that cancers will have a greater need for growth factors than normal cells or be dependent upon a tumour specific factor.

FUTURE PERSPECTIVES

Recent years have seen major changes in the nature of endocrine treatment for breast cancer. Endocrine surgery which was both irreversible and held substantial risk of morbidity has been largely superceded by drugs which either diminish the availability of oestrogen or its action within breast tissues. Because these agents are effective and specific it has been possible to reduce greatly side effects not associated with hormone deprivation. For the immediate future there are also novel drugs in the pipeline—purer antioestrogens, suicide aromatase inhibitors and depot preparation of new LHRH analogues. All these agents offer the promise of non-toxic, long-acting hormone deprivation which can be

expected to simplify treatment and cause the minimum discomfort to the patient. Whilst these newer drugs will be first administered to patients with overt metastatic disease, if the properties of minimal morbidity are realized, then a wider application must be considered. The majority of women present with cancer evidently confined to the breast but the natural history of the disease suggests most patients already have occult distant metastases and would benefit from systemic therapy given to eliminate micrometastatic tumour. The advent of non-toxic hormone treatment makes such adjuvant therapy much more acceptable to women who can see no evidence of tumour following local treatment of their breast.

The identification of factors which predict for endocrine dependence in individuals is also an important objective. Only the minority of patients have hormone-sensitive tumours and whilst newer endocrine measures limit exposure to unacceptable side-effects, non-selective administration of endocrine therapy is inefficient and delays the implementation of potentially more beneficial treatment in most patients. This may be an important consideration, if alternative treatment is chemotherapy which dogma suggests is most effective when tumour burden is low. Optimal management must therefore profit from the accurate identification of responsive patients for particular treatments.

Whilst low toxicity hormonal regimes and accurate identification of hormone responsiveness represent major advances and should not be under estimated, it has to be emphasised that hormone deprivation in most cases produces cytostatic rather than cytocidal effects and that, as tumours evolve under the selective pressure of hormone deprivation, an increasing proportion of breast cancers become resistant to endocrine measures. This means that even after successful endocrine treatment most patients still die of their disease but with tumours whose growth is evidently no longer dependent upon oestrogen.

If therefore survival rates are to be markedly improved with hormone therapy, we must learn how to kill more tumour cells whilst they are held in antihormone-induced quiesence and simultaneously prevent the emergence of clones of endocrine-resistant cells. In these areas we have to look to greater knowledge of the molecular events underlying such processes. With this understanding it may be possible to identify mechanisms which are under the control of hormones in endocrine sensitive tumours but regulated by other factors in more autonomous cancers. Using this approach agents could be developed which would be effective irrespective of tumour hormone sensitivity. The recent research on autocrine/paracrine secretion of growth factors by tumour cells, some of which are under hormone control, may be relevant in this respect.

The final perspective must be that although this review has been concerned with endocrine management of breast cancer, the point has to be made that knowledge deriving from research into (i) the endocrinology of established breast tumours and (ii) drugs used as therapy for relatively advanced disease may help the understanding of earlier stages of the disease process such as the transformation of high risk normal breast epithelium into cancer cells and the transition from non-invasive to invasive cancer. In this way the twin goal of prevention and cure of breast cancer may be more quickly realised.

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