virus and retroviruses. Transfusions also pose issues associated with cost and scarcity. Thus, I conclude today, as we did in 1987 [15], that "avoidance of unneeded tranfusions or use of erythrocytes in preference to whole blood is sound medical practice for a number of reasons ... even if a decrease in tumour recurrence has not been established".

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Novel Approaches to the Endocrine Therapy of Breast Cancer

M. Dowsett

INTRODUCTION

THE DEPENDENCE of some breast cancers on ovarian function for their continued growth has been recognised for nearly a century [1]. This led to the successful development and application of several different treatments based on depriving the breast carcinoma of its oestrogenic stimulation. Some of these, such as tamoxifen, have a well-established place in therapy while others are still in preclinical or early clinical stages. More recently the recognition that breast carcinomas are subject to many other stimuli that influence their growth has led to the development of new groups of compounds which have mechanisms of action other than oestrogen deprivation.

Endocrine treatment of breast cancer was established as a major therapeutic option in the early 1970s with the availability of tamoxifen which remains the dominant drug in the field [2]. The successful use of tamoxifen over the past 20 years might argue against the value of developing other agents directed at oestrogen deprivation. However, there are several observations to support such research: (a) tamoxifen is not effective in all patients with oestrogen and progesterone receptor positive tumours, which would be expected to be responsive to oestrogen deprivation; (b) some patients who do not respond to tamoxifen do respond to some other modes of endocrine therapy; (c) at relapse, responders to tamoxifen frequently respond to other types of endocrine therapy; and (d) many (probably the majority) of the pharmacological effects of tamoxifen are oestrogenic, which argues against tamoxifen's mechanism of action solely as an oestrogen antagonist.

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ENDOCRINE THERAPIES

New anti-oestrogens

The side-effect profile of tamoxifen is excellent to the degree that the drug has been accepted by the UK Coordinating Committee for Cancer Research for study as a chemopreventive for breast cancer in healthy women following encouraging feasibility studies [3]. New anti-oestrogens are therefore likely to be of value only if they have improved efficacy or are effective in patients in which tamoxifen is ineffective, possibly because tamoxifen is a mixed agonist/antagonist. For antitumour effects, agonist activity is undesirable and pure antagonists may be more effective in suppressing breast cancer growth. The mainstream approach to this problem has been the synthesis of triphenylethylene analogues, such as toremifene and droloxifen, with close structural similarity to tamoxifen [4, 5]. In laboratory and animal experiments, however, none of these compounds is purely anti-oestrogenic. It would therefore be surprising if such compounds target different cells and have a different clinical profile from tamoxifen.

Zindoxifene is an anti-oestrogen with a different structure, being an acetylated indole. However, it also has mixed agonist and antagonist activities and although studies with carcinogen-induced rat mammary tumours were encouraging, none of 25 patients showed an objective response in a phase I/II study [6].

An exciting new series of oestradiol derivatives are pure anti-oestrogens *in vitro* and in animal studies [7]. The modification is by linkage of an aliphatic chain at the 7α position. The stability of the modification is all important since cleavage could lead to the release of oestradiol, the biologically active oestrogen. The preclinical data are encouraging—some of these compounds suppressed the growth of both oestrogen-dependent breast cancer cell lines *in vitro* and induced rodent mammary carcinomas more effectively than tamoxifen [8, 9]. Clinical studies are awaited with great interest.

However, the close similarity of the compounds to oestradiol may pose a problem in application since they will probably be rapidly cleared as ineffective metabolites via the same route as oestradiol. The pharmacology of tamoxifen is complex, with wide biochemical and physiological effects. Some of these effects may be involved in the efficacy of the compound, yet may not result from oestrogen antagonism (e.g. calmodulin inhibition [10]). Thus a pure anti-oestrogen might in fact be less efficacious than tamoxifen. The oestrogen agonist effects of tamoxifen on bone and plasma lipids have also added to its acceptability for long-term adjuvant therapy and prophylactic trial. A pure antagonist would undoubtedly prove to be a valuable biochemical probe in breast cancer research, but the clinical future of the concept whilst exciting is uncertain.

Aromatase inhibitors

Aromatase inhibition is an alternative to antagonism in depriving the tumour of oestrogen stimulation. Anti-oestrogens have one theoretical advantage over aromatase inhibitors: the antagonists would be expected to oppose the effects of any exogenous oestrogens, such as phyto-oestrogens in the diet and of some adrenal androgens which exhibit oestrogenic activity *in vitro*. However, the clinical significance of these two potential oestrogenic stimuli is unknown. Indeed full blockade of oestrogen synthesis by a potent aromatase inhibitor may provide a more complete reduction of oestrogen stimulation than that obtained with an antagonist, which would need to be given at a dose sufficient to saturate the receptor to achieve maximal deprivation.

The demonstration that aminoglutethimide was effective in breast cancer as an aromatase inhibitor [11, 12] led to much effort to derive an inhibitor with better specificity and without the clinical side-effects of aminoglutethimide. A series of these inhibitors has been developed and is most readily subdivided by the steroidal or non-steroidal nature of the compounds. Each subgroup has a representative in phase III trial.

The steroidal compounds (e.g. 4-hydroxyandrostenedione [CGP 32349], MDL 18962, FCE 24304, SH 489) are analogues of the substrate androstenedione, and are mainly "suicide inhibitors" that are converted by aromatase to a reactive intermediate which irreversibly inactivates the enzyme. Such compounds have the potential for exquisite specificity since they can be considered as a pro-drug whose active derivative is yielded only after interaction with its target enzyme. There is now considerable experience with 4-hydroxyandrostenedione, which is effective both endocrinologically and clinically [13, 14]. The only significant side-effects have been reactions at the injection site when the drug is given intramuscularly.

Of the non-steroidal compounds, CGS 16949A is not totally selective for aromatase since it suppresses plasma aldosterone levels as well as oestrogen levels [15]. Three other inhibitors, R76713, CGS 20267 and pyridoglutethimide, also show clinical promise.

Which of these compounds is likely to be in use as the mainline aromatase inhibitor in 5 years' time? The selection may depend on the drug's ability to achieve total suppression of aromatase activity in all patients with insignificant side-effects. With these demands most aromatase inhibitors may fall by the way-side. However, another question must be answered: to what degree must aromatase be inhibited to achieve maximum clinical benefit? Submaximal inhibition may turn out not to be suboptimum treatment, in which case we may have a plethora of adequate drugs from which to choose.

Gonadotropin releasing hormone analogues

Several gonadotropin releasing hormone (GnRH) agonists are now established as acceptable alternatives to ovariectomy in premenopausal breast cancer patients, with about one-third of unselected patients with advanced disease achieving an objective response. Indeed the generic terms of "medical castration" or "medical ovariectomy" have frequently been used (in fact a misnomer, since "selective hypophysectomy" is more correct if an anatomical analogy is required). The steroidal changes with GnRH agonists appear virtually identical to those after castration after an initial stimulatory phase. These compounds have been studied in post-menopausal women but efficacy was poor [16]. The few responses recorded have been suggested to result from either a direct effect of the agonist or suppression of ovarian androgen synthesis with a consequent fall in oestrogens [17].

It is likely that GnRH antagonists will soon enter clinical trial in breast cancer. The advantage of such antagonists is that, unlike the agonists, they do not have an early stimulatory phase in their action, and therefore achieve steroidal suppression more rapidly. However, the clinical impact of a change from agonists to antagonists is likely to be small. There are few reports of significant disease flare being associated with agonist usage and a delay of perhaps two weeks in achieving oestrogen suppression probably matters little in a disease that is generally slowly progressive.

Several critical questions remain about the use of GnRH analogues: (a) in metastatic disease should treatment persist after relapse; (b) in adjuvant therapy should treatment continue until expected natural menopause; and (c) what is the comparable effectiveness of ovarian ablation (surgical, radiotherapeutic or medical) and cytotoxic chemotherapy in the adjuvant treatment of premenopausal breast cancer?

Antiprogestins

These are a new, potentially valuable group of agents whose action appears to be independent of oestrogens. The first antiprogestational agent (mifepristone) has growth inhibitory effects on human breast cancer cells *in vitro* [18] and in carcinogen-induced rat mammary tumours [19]. Short-term antitumour effects have also been noted in the small clinical studies reported so far. Mifepristone has become a controversial drug because of its use as an abortifactant, but it also has antiglucocorticoid activity which may complicate its use. However, the antiglucocorticoid action is less apparent with onapristone and ZK 112993.

The mechanism of the antitumour effect of the antiprogestins is unknown. Animal studies suggest a direct effect on tumour cells mediated via progesterone-receptors. The anti-proliferative effect may be due to induction of terminal differentiation which ultimately leads to terminal cell death [20]. Clinical studies of this finding are awaited with great interest.

Vitamin D analogues

It has been known for many years that 1,25 $(OH)_2$ Vit D_3 (calcitriol) has a major endocrine role in calcium homoeostasis. More recently it has been recognised that 1,25 $(OH)_2$ Vit D_3 stimulates differentiation and reduces proliferation in many cell systems. These observations have led to preliminary clinical studies of vitamin D or its analogues in a number of malignant diseases, including breast cancer in which 1,25 $(OH)_2$ Vit D_3 receptors are frequently present [21]. The analogue MC903 is a less potent hypercalcaemic agent than the natural hormone, whilst similar potency as a differentiating agent is maintained [22]. The effectiveness of vitamin D or currently available analogues may be reduced by their rapid metabolism but they or other more stable analogues provide exciting possibilities for the treatment of several tumour types in the future.

Somatostatin analogues and bromocriptine

Antiprolactin therapy has been disappointing in breast cancer. The combined use of the somatostatin analogue, sandostatin, with bromocriptine to suppress growth hormone and prolactin has been studied in small numbers of patients by several groups. In general, efficacy has been low but at least one response has been noted [23]. The patients had all been heavily pretreated. Study of the combination earlier in metastatic disease is merited.

Combination treatment

The combination of different types of endocrine therapy in breast cancer treatment has not been explored widely. Animal studies indicate this approach to be potentially valuable [24] but the clinical reports of the combination of tamoxifen with aromatase inhibitors and other endocrine agents have not been encouraging [25]. Sometimes, drug interactions may make certain combinations (such as aminoglutethimide with danazol [26]) disadvantageous. The many new compounds that are becoming available provide the opportunity to pursue this area further. This research should be on the basis of an established rationale, with drugs whose pharmacology is well understood and in conjunction with detailed pharmacoendocrine studies to ensure an absence of significant drug interaction.

The combination of tamoxifen with a GnRH agonist in premenopausal breast cancer is an approach to complete oestrogen deprivation that is under examination [27]. The agonist activity of tamoxifen suggests that some oestrogenic stimulation may persist with that combination, and the use of a potent aromatase inhibitor with a GnRH agonist is an alternative worth examining [28].

Endocrine chemoprevention

I have mentioned trial use of tamoxifen as a chemopreventive in women between the ages of 45 and 65. Another approach which is under consideration for premenopausal women is the development of a contraceptive that also has a protective effect on the breast. Experimental support for this concept derives from the finding that the progestogen, gestodene (which is in some oral contraceptives), has an antiproliferative effect on breast cancer cells *in vitro* [29].

CONCLUSION

Careful exploration of each of the approaches discussed may be realistically expected to define agents or combinations of agents that will be of significant benefit in the treatment of breast cancer patients. Certain of the compounds hold the promise of being valuable probes for the study of breast cancer biology *in vivo*. It is essential that close relationships are established between oncologists, pathologists and biologists during the clinical study of these therapies to ensure that this possibility is realised. Thereby a rationale for the development of yet further agents to manipulate endocrine growth control of breast cancer may be established.

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Science Watchers

Mammalian Sex Determination

Mark S. Palmer

TWO RECENT reports in *Nature* [1, 2] have suggested that the elusive sex-determining gene (or more properly that for the testis determining factor [*TDF*]) may finally have been identified, with the description of a new gene, *SRY*, from the smallest region of the human Y chromosome known to be sex-determining (hence sex-determining region on the y chromosome). The history of the quest for *TDF* has been somewhat chequered, and *SRY* is the fourth candidate sequence to be described. It is therefore understandable that the two groups of investigators have stressed that while *SRY* is now the best candidate sequence, the proof must await functional studies of sex-reversed transgenic mice or mutational analysis of XY females. Indeed the possibility of other coding sequences near to *SRY* has not been ruled out.

The Y chromosome has been known to be sex-determining in man since 1959 following observations on the karyotype of a male with Klinefelter syndrome [3] and a female with Turner syndrome [4]. The Y chromosome has since been subjected to extensive genetic and molecular analysis in an attempt to pinpoint the genes involved. An early sequence thought to play a role in sex-determination was Bkm (banded krait mini satellite) DNA, a satellite DNA containing mostly simple GATA–GACA tandem repeats. Bkm sequences, as well as showing sex-specific localisation in the snake, were concentrated on the mouse Y chromosome, particularly on the sex-determining region (*SXR*); however, they were poorly represented on the human Y chromosome [5]. This left the stage open for the most enduring candidate, the male-specific minor histocompatability antigen, H-Y, originally described as a male-specific transplantation antigen on male skin grafts in certain strains of inbred mice. The most fundamental requirement of a sex-determining gene on the Y chromosome is that its presence should be necessary for the development of the indifferent fetal gonad into a testis. (After testicular development has been initiated further sexual development is under hormonal control.) The finding of male mice and men with normal testicular development but lacking H-Y antigen meant that this hypothesis also had to be abandoned [6, 7].

The next sequence to be considered, *ZFY*, was identified by a molecular analysis of that part of the Y chromosome inherited by XX males. Ordinarily during male meiosis the X and Y chromosome pair and undergo an obligatory recombination

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