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INHIBITORS OF STEROIDOGENIC ENZYMES FOR THE TREATMENT OF BREAST CANCER

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Summary—The most important mitogen for human breast cancer is oestrogen. Since oestrogens are synthesized via a protracted series of enzymic conversions from cholesterol, there are many potential targets for inhibition which could theoretically lead to suppression of oestrogen synthesis. However, inhibition of many of these targets is complicated by a resultant interference in the synthesis of other steroids, particularly glucocorticoids. This results in inhibitors of aromatase being the most rational choice for oestrogen suppression in breast cancer patients. Several aromatase inhibitors are in clinical usage. It is important that the clinical effectiveness of these is compared with that of the antioestrogen, tamoxifen.

INTRODUCTION

The endocrine treatment of breast cancer is based on the observation that a proportion of breast carcinomas are dependent on oestrogen for their continued growth. Surgical techniques were used for many years to remove glands responsible (either directly or indirectly) for oestrogen production. Hypophysectomy or adrenalectomy were performed in postmenopausal women, whilst in premenopausal women ovariectomy remains a frequently performed therapeutic manoeuvre. Nowadays, oestrogen deprivation is more frequently achieved by medical treatment: in premenopausal women GnRH agonists or the antioestrogen tamoxifen are used, whilst in postmenopausal women the alternative to tamoxifen is an inhibitor of oestrogen synthesis. These enzymes inhibitors have not found application in the premenopausal group, since the agents used so far have not been sufficiently potent to overcome the effects of increased gonadal drive which results from decreased feedback inhibition.

ANTAGONISTS OR ENZYME INHIBITORS?

There are theoretical advantages and disadvantages to the use of both antagonists and inhibitors of steroidogenic enzymes involved in oestrogen synthesis (Fig. 1). In addition to true oestrogens (i.e. steroids which contain an aromatic A-ring) there are endogenous compounds which have oestrogenic potential, the best characterized of these being androstenediol. There is also evidence that the diet may contribute oestrogen-like compounds, e.g. phytooestrogens, which may contribute to the overall oestrogenic stimulation to a breast carcinoma. An oestrogen antagonist would be expected to oppose all of these routes of stimulation, whilst an enzyme inhibitor would not suppress exogenous stimuli and would only have potential for suppressing androstenediol, if it interacted with an enzyme prior to the androstenediol in the route to oestrogen synthesis (see Fig. 2). However, whilst an antioestrogen would appear to have at least a theoretical advantage in this regard, no pure antagonists are currently available for breast cancer treatment. Tamoxifen and each of the other triphenylethylene derivatives have a partial agonist activity. The significance of the agonist activity on efficacy is unknown but it is notable that in some animal systems tamoxifen has been shown to support tumour growth after the tumour has initially regressed. Enzyme inhibitors exist which lack significant interaction with the oestrogen-receptor (and any other steroid-receptor) and thus possess no oestrogen-agonist activity.

Pure steroidal antioestrogens are expected to shortly enter early clinical trials. ICI 164384 is

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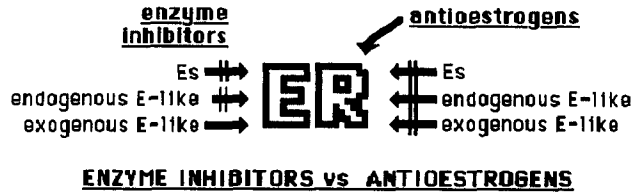

Fig. 1. Oestrogen deprivation by enzyme inhibitors and antioestrogens. In contrast to the inhibitors, antioestrogens should oppose all oestrogenic signals but may themselves have agonist activity.

an example of this group of compounds [1]. It is an analogue of the oestradiol molecule with a long aliphatic side-chain at the 7α position. The application of these compounds is awaited with interest but there are a number of obstacles for them to clear: these include the development of a pharmacologically effective and clinically acceptable formulation which delivers enough drug to exert a sufficient antagonistic effect to achieve tumour remission.

Currently, each of these relative advantages and disadvantages remain theoretical. Indeed, it is not currently known whether a complete deprivation of oestrogen is necessary to achieve a maximal degree and duration of tumour regression. To determine this and to test their theoretical comparisons, studies are required to compare the antagonists and enzyme inhibitors for their effects on oestrogen-dependent gene expression and most importantly on tumour cell mitosis. Maximum information would be derived from these investigations if they could involve sequential measurements on tumours in patients in randomized clinical trials of enzyme inhibitors and antagonists.

INHIBITORS OF STEROIDOGENIC ENZYMES FOR OESTROGEN DEPRIVATION

The inhibition of any of several enzymes involved in oestrogen synthesis might be expected to result in a degree of oestrogen deprivation (Fig. 2). Since the application of these inhibitors is in postmenopausal women and the major organ involved in synthesis of androgenic oestrogen precursors is the adrenal, the inhibition of each of the enzymes involved in androgenesis would also tend to reduce cortisol levels. The effect of this is to lead to increased drive to the adrenals which would tend to oppose the blockade of the target enzyme. If a specific inhibitor of 17,20 lyase were available this would not affect cortisol synthesis. However, 17,20 lyase activity is integrated in a single enzyme complex with 17α-hydroxylase and all inhibitors of the lyase activity which have been described also inhibit the 17-hydroxylase. An example of such an inhibitor is ketoconazole. This has not been explored extensively in breast cancer patients because of its toxic side effects. In a small study we demonstrated that suppression of plasma

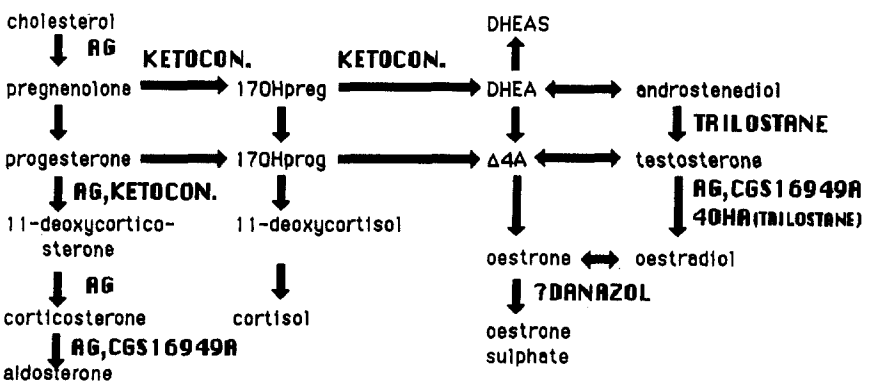

Fig. 2. Inhibitors of steroidogenic enzymes for breast cancer treatment. The diagram shows the multiple sites of inhibition of the different agents. AG, aminoglutethimide; ketocon, ketoconazole.

oestradiol levels was achieved but this was not as great as that seen with some aromatase inhibitors and no effect on oestrone was observed [2].

Trilostane is an inhibitor of 3β-hydroxysteroid-dehydrogenase-isomerase, which it is claimed also inhibits aromatase with low potency [3]. The tendency of this compound to suppress cortisol synthesis has led to its combined use with glucocorticoid. In this combination some clinical efficacy has been noted [4].

However, the inhibition of aromatase, the enzyme responsible for converting androgens to oestrogens, is theoretically a more logical target for inhibition and this has been exploited extensively. Aromatase is the last enzyme involved in oestrogen synthesis, such that its specific suppression should not affect the synthesis of any other steroids. It is likely that aromatase inhibition would be more effective than suppression of precursors of the enzyme inhibitors of other enzymes. In postmenopausal women aromatase activity is present almost exclusively in peripheral non-glandular tissues. There is no known feedback control of this activity to oppose the inhibition. In addition since only 1–4% of androstenedione and $<1\%$ testosterone are metabolized by aromatase in postmenopausal women there is unlikely to be any build-up of precursor prior to the enzyme blockade.

Unfortunately, the first aromatase inhibitor to be used widely in breast cancer patients, aminoglutethimide, had a wide spectrum of inhibitory effects on other cytochrome P_{450} dependent steroid hydroxylases such as 11β-, 18- and 21-hydroxylases as well as the 20-hydroxylase activity involved in cholesterol side-chain cleavage [5]. Whilst these side-effects are undesirable and lead to the drug's combined use with glucocorticoid it has been argued that another effect of aminoglutethimide—the enhancement of the clearance of oestrone sulphate—is advantageous and may contribute to the efficacy of the drug [6]. Aminoglutethimide remains the only aromatase inhibitor in widespread usage but it is likely to be superseded in the near future by one or more of a large number of aromatase inhibitors which are under preclinical or clinical evaluation.

Two of these inhibitors 4-hydroxyandrostenedione (Lentaron) and CGS 16949A (Fadrozole) have been subject to detailed characterization in breast cancer patients under clinical trial. As steroidal and non-steroidal inhibitors, respectively, they are representatives of the two major groups of new inhibitors.

4-Hydroxyandrostenedione is a k-cat or suicide inhibitor which can be shown *in vitro* to irreversibly inactivate aromatase [7]. This type of inhibitor has the potential for high specificity and prolonged effectiveness. 4-Hydroxyandrostenedione is pharmacologically and clinically effective by both the parenteral and oral routes, although the former has been studied to a much greater extent [8–11]. Plasma levels of oestradiol are suppressed markedly and consistently by 250 and 500 mg every 2 weeks. Oestrone levels are similarly suppressed, and no significant effect on androgen, gonadotrophin, cortisol, aldosterone, TSH or SHBG levels has been noted. Suppression of aromatase by 4-hydroxyandrostenedione has been estimated by *in vivo* radioactive infusion techniques. The 250 and 500 mg/2-week doses were found to inhibit aromatase by a mean ($\pm$SEM) 84.9 ± 2.0 and $91.9 \pm 1.0\%$, respectively.

CGS 16949A is about 300 times more potent *in vitro* than aminoglutethimide [12]. It suppresses oestradiol and oestrone levels significantly at doses as low as 0.3 mg b.d. (twice daily) [13]. Progressively greater suppression is achieved by 1 and 2 mg b.d. Clinical responses have been noted at these doses but both lead to a reduction in the plasma levels of aldosterone which are associated with statistically significant changes in the electrolyte balance [14]. These are only minor in degree and there may be no clinical significance in most patients. The suppression appears to be due to inhibition of the corticosterone methyloxidase type II enzyme, the terminal step in aldosterone biosynthesis since the ratios of plasma 18-hydroxycorticosterone/aldosterone and of urinary 18-hydrotetrahydroaldosterone/tetrahydroaldosterone are increased in patients treated with high doses (8 mg b.d.) of CGS 16949A [15]. Radioactive tracer measurements *in vivo* indicated that in 8 patients on 1 mg b.d. aromatization was decreased by $82.4 \pm 2.9\%$. In 3 of these patients aromatization was also measured at 2 mg b.d. and inhibition was increased from 80.4 ± 6.8 to $92.6 \pm 2.8\%$ [16].

A series of other inhibitors are approaching the point of detailed clinical and pharmacological evaluation. This should allow the selection of a specific inhibitor which lacks clinical side-effects and is sufficiently potent to approach complete aromatase blockade. The assessment and optimization of these inhibitors depends on our ability

1. 100% inhibition
2. aromatase inhibition vs pure antioestrogens
3. high sensitivity, definitive assays
4. other indications;
   - ovulation induction/control
   - endometrial cancer
   - ? BPH

Fig. 3. Future developments/questions in aromatase inhibition.

to quantify accurately the residual aromatase activity and resultant oestrogen (Fig. 3). This requires the development of yet more sensitive, highly-specific assays. The availability of a potent aromatase inhibitor with insignificant side-effects will allow an assessment to be made of the potential of such compounds in other indications such as endometrial cancer and benign prostatic hypertrophy. Another virtually unexplored area with aromatase inhibitors is control and induction of ovulation. The short half-life of some of the non-steroidal compounds may give them an advantage over the currently used antioestrogens which may persist in the tissues after ovulation and have a detrimental effect on ovarian luteal and endometrial secretory function.

Another steroidogenic enzyme which has been targeted for inhibition as a potential treatment for breast cancer is oestrone sulphatase. This is on the basis that oestrone sulphate may act as a major precursor for the synthesis of non-conjugated, biologically active oestrogen. The evidence for this is mixed and no sufficiently potent or specific inhibitor has been described to evaluate this concept *in vivo*. Danazol is frequently quoted as a sulphatase inhibitor [17] but its potency is low and it has a multitude of other pharmacological effects. Aromatase inhibition leads to the suppression of oestrone sulphate and it seems unlikely that sulphatase inhibition alone is likely to be more effective than aromatase inhibition. However, given that suppression of circulating oestrogens including oestrone sulphate is not complete with the aromatase inhibitors used as yet the combination of inhibitors of both aromatase and oestrone sulphatase is an attractive concept.

CONCLUSION

Inhibitors of some steroidogenic enzymes have been shown to be clinically effective in breast cancer. Inhibitors of aromatase are finding wide clinical utility. Clinical studies are awaited to determine the comparative effectiveness of antagonists and inhibitors in oestrogen deprivation.

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