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CLINICAL DEVELOPMENT OF AROMATASE INHIBITORS FOR THE TREATMENT OF BREAST AND PROSTATE CANCER

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Summary—Numerous aromatase inhibitors are under development for breast cancer treatment. The major aims are to obtain a drug which at its dose of maximum efficacy has no effect on other endocrine systems, has no clinical side-effects and is convenient to administer. During the early clinical stages of development detailed endocrine and pharmacokinetic analyses are a valuable aid in the establishment of a drug's selectivity and its optimum dose, route and frequency of administration. The optimal dose may be defined as the minimum that will achieve maximal and sustained suppression of aromatase activity. This has generally been measured indirectly by comparing the suppression of plasma oestrogen levels at a selection of dosages. This approach has major advantages in speeding dose selection for therapeutic clinical trials. However, it also has some disadvantages including the unproven assumption that clinical response has a direct relationship with the degree of oestrogen suppression. In addition there are technical difficulties of analysis, of wide variability in endocrine response between patients and of demonstrating oestrogen suppression to be equivalent between doses (necessary to show maximal suppression). The direct measurement of aromatase inhibition *in vivo* by isotopic infusion analysis provides support to these indirect estimates. Its value is shown by our recent results with CGS16949A. The additional value of collating pharmacokinetic and endocrine measurements is apparent from our investigations of 4-hydroxyandrostenedione (4-OHA) and pyridoglutethimide. A consideration of our experience with these inhibitors may be helpful in directing the development of future agents.

Whilst the value of aromatase inhibition in breast cancer is established its value in prostatic cancer is in doubt: we have found that 4-OHA is only poorly efficacious in advanced prostatic cancer.

INTRODUCTION

The development of inhibitors of aromatase for the clinical manipulation of numerous oestrogen-dependent processes is an attractive option since current knowledge indicates that the synthesis of all oestrogen occurs through this route. In addition, since there are no other physiologically active products as a result of oestrogen metabolism, the deprivation of these steroids should only have consequence for oestrogen-sensitive tissues.

It has long been considered that many breast carcinomas are dependent on oestrogen for their continued growth. In the premenopausal woman the major source of oestrogens is ovarian. Suppression of plasma oestrogens from this source by the aromatase inhibitors aminoglutethimide (AG) and 4-hydroxyandrostenedione (4-OHA; CGP 32349, CIBA-Geigy) has been largely unsuccessful [1, 2]. This is probably due to the increase in gonadotrophin drive which results from reduced negative feedback of plasma oestrogens. For this reason, surgical or radiation-induced ovarian ablation and LHRH agonists are likely to remain the mainstay of oestrogen deprivation in premenopausal breast cancer patients. The combination of an aromatase inhibitor with ovarian ablation is an approach to complete oestrogen withdrawal which is endocrinologically effective [3] but remains to be tested in comparative clinical trials.

The postmenopausal ovary continues to produce androgens [4] but is devoid of aromatase and the conversion of both the ovarian and adrenal androgens to oestrogens occurs in peripheral tissues, most notably the stromal cells of subcutaneous fat. Importantly there does not appear to be a substantial or effective feedback control of oestrogen synthesis in these women, such that oestrogen deprivation by aromatase inhibitors is "unopposed".

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The clinical effectiveness of aromatase inhibition was first demonstrated with AG. This drug was initially employed in breast cancer in combination with a corticosteroid in an attempt to achieve a medical adrenalectomy. It has since been recognized that the adrenal effects of AG are detrimental to the overall aim of oestrogen deprivation since its inhibition of 11β-hydroxylase results in increased adrenal androgen production [5, 6]. The oestrogen suppressive activity of AG appears to be almost entirely due to its inhibition of aromatase [7]. Its clinical effectiveness without the combined use of a glucocorticoid [5, 8] demonstrated that aromatase inhibition was a viable therapeutic option in postmenopausal breast cancer patients.

There are several disadvantages to aromatase inhibition with AG. It is a relatively non-specific cytochrome *P*450 inhibitor and it should be used in combination with a glucocorticoid for therapeutic safety [9] and effectiveness as an oestrogen suppressant [10]. Use of AG may also lead to one or more of a number of clinically significant side-effects (e.g. nausea, lethargy, ataxia, rash, blood dyscrasias). AG has therefore been seen as a prototype aromatase inhibitor and for the last 10 yr many research and development groups have pursued the objective of the ideal aromatase inhibitor. This may be defined as a drug which:

(i) suppresses oestrogen synthesis maximally *at a dose* at which it:
(ii) is highly selective for the aromatase target; and
(iii) lacks significant clinical toxicity.

It would also be advantageous for it to be possible to formulate the drug as convenient to administer for both the clinician and the patient.

Over the last 10 yr we have examined five prospective candidates as ideal aromatase inhibitors. These are listed in Table 1 together with any points which are against their acceptance as the *ideal* inhibitor. Although each has unfavourable points, in general these are minor and other than miconazole each of these has some clinical utility. Indeed 4-OHA approaches the ideal but it has minor androgenic activity (which has only been noted in animals [11] and in orally-treated patients [12]) and it is currently only available in a parenteral form (once every 2 wk by intramuscular injection) which some may consider an inconvenient formulation. We are therefore in a situation in which further inhibitors will shortly be presented for clinical development. It is the aim of this article to examine critically the approaches which we have taken in the development of the earlier inhibitors with a view to optimizing the procedure for these newer compounds.

Table 1. Aromatase inhibitors used in pharmacological–clinical studies in breast cancer by our group and some problems which make them non-ideal.

Inhibitor	Problems
AG ± HC	Non-specific, co-use with HC Clinical toxicity
4-Hydroxyandrostenedione	Parenteral administration androgenic?
CGS16949A	Aldosterone suppression
"Pyridoglutethimide"	Potency Non-ideal pharmacokinetics
Miconazole	Potency Clinical toxicity

AG, Aminoglutethimide; HC, hydrocortisone

A PHARMACOLOGICAL APPROACH

An advantage to the development of agents which are aimed at achieving hormone deprivation is that it is usually possible to measure the desired pharmacological change (e.g. suppression of plasma oestrogens). This is quite different from the situation with antagonists of hormone action when hormonal changes in body fluids are usually not directly applicable as a measure of mechanistic effectiveness. Thus measurement of circulating oestrogen levels has been the main measure of the effectiveness of aromatase inhibitors. In breast cancer the availability of such a measure is particularly important since the effectiveness of any hormonal therapy will undoubtedly be highly variable through the population: even groups selected on the basis of positive steroid receptor status are likely to demonstrate only a 50% response. This means that if development of a drug is based on its clinical efficacy, comparative clinical trials of many tens and probably hundreds of patients would be required to select the most appropriate dose, route and interval of treatment. The use of oestradiol measurements in association with carefully designed protocols can allow a far more rapid achievement of the optimal therapeutic regime. The lynchpin in this approach is that there is a relationship between these oestrogen measurements and the efficacy of treatment. Currently, however, this is an assumption the investigation of which is ethically difficult since it inevitably leads to the deliberate undertreatment of a group of patients. In general it has been accepted that the advantages of a pharmacological approach are such as to outweigh this uncertainty.

Probably the most valuable approach to take is to combine the measurement of plasma oestrogen levels with those of the inhibitor. We used this approach at an early stage of development with 4-OHA [13] and more recently have performed similarly valuable investigations with "pyridoglutethimide" (PG), an analogue of AG which has been developed by Dr Jarman at the Institute of Cancer Research [14]. An example of the work with PG is shown in Fig. 1. Oestradiol and PG measurements were made in serum at frequent intervals in patients after a single dose and after 5 daily doses of 1000 mg PG. It can be seen that the oestradiol levels in this patient recovered more rapidly after repeated dosing than after a single dose. This is explained by the circulating levels of PG which were about 5 μg/ml, 24 h after the single dose but were undetectable 24 h after repeated doses, because of induced metabolism. This type of approach allows early definition of the minimum effective serum concentration of drug. Thereafter pharmacological modelling can rapidly speed the achievement of an optimal therapeutic regime.

One of the aims for the ideal drug is that it should lack significant endocrine or clinical side-effects. It should be recognized that such undesirable effects are generally dose-related as shown diagrammatically in Fig. 2. The aim is that any side-effects should be of the B- or preferably C-type. As indicated above each of the drugs investigated has at least one problem which is of the A-type. To minimize the importance of such side-effects one is therefore placed in the position of defining the minimum dose to achieve maximal suppression, the point indicated by the vertical line in Fig. 2. The approach to this has been almost exclusively to compare the oestrogen suppressive effects of the inhibitor

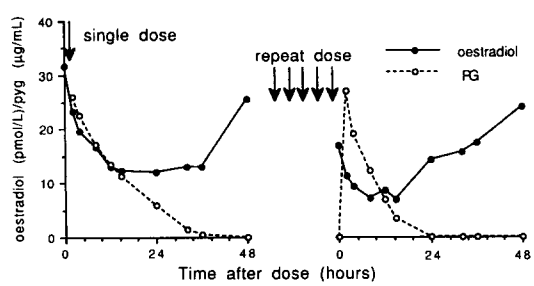

Fig. 1. The plasma oestradiol suppressive effects and pharmacokinetics of "pyridoglutethimide" (PG) after a single dose and 5 repeated doses of 1000 mg in a single patient. The pattern is similar for the other four patients studied under this protocol. The zero hours sample was taken just prior to the dose. No drug was administered during the sampling period.

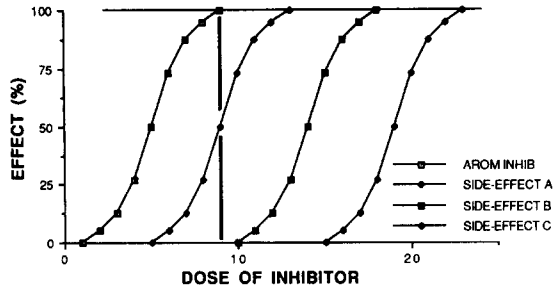

Fig. 2. Hypothetical relationship between suppression of oestrogen synthesis and potential side-effects (clinical or endocrine) of aromatase inhibitors. The vertical line indicates the dose of maximum inhibition of oestrogen synthesis. The ideal is that any side-effects should be of type-C.

at various doses. This approach can be valuable but it brings with it a series of problems and questions which should be addressed before the design of protocols and during the interpretation of results. The more important factors are listed in Table 2 and each is dealt with briefly below.

Firstly, the suppression of oestrogens may be monitored in the levels of one or more of several oestrogens (oestrone, oestradiol, oestriol, oestrone sulphate, oestrogen glucuronides, total oestrogens) in a series of body fluids (blood, urine, saliva, tumour homogenate). In principle a pure aromatase inhibitor would be expected to affect each of these oestrogen/fluid combinations in a proportionally equal manner. In practise a different answer is likely to be obtained with each combination, at least partly because of analytical problems in measuring postmenopausal oestrogen levels.

We have taken the view that the most appropriate measurements are plasma oestradiol levels on the principle that oestradiol is biologically the most potent oestrogen and that plasma is biologically the most relevant fluid: tumour homogenates and nipple aspirates are not sufficiently readily available for pharmacological utility. This choice was also made on the grounds of the availability of a highly-sensitive and specific oestradiol assay [13]. The ability to

Table 2. Important factors to consider in the pharmacological development of aromatase inhibitors

1. Measurement of oestrogen suppression:
   (a) which oestrogen, which fluid?
   (b) logarithmic basal distribution,
   (c) which parameter of suppression?
   (d) what is maximal suppression?
   (e) variable pharmacokinetics
   (f) variable response
   (g) compliance
   (h) concomitant therapy
2. Statistics:
   requirement of equivalence of suppression
   NOT lack of statistical difference

measure to a sensitivity of 10% of the mean normal level of the analyte in question is a prerequisite of accurate characterization of the pharmacological effects of aromatase inhibitors.

The mathematical parameter by which suppression of oestrogen levels is judged is uncertain. Comparisons may be made of absolute on-treatment levels, of on-treatment levels as a percentage of pretreatment values or the absolute reduction in levels during-treatment. The logarithmic normal distribution of oestradiol levels makes this an important issue but one which is difficult to answer since the "dose" relationship between oestrogen suppression and clinical effectiveness has not been characterized. The recruitment of groups for comparison with similarly distributed pretreatment levels to some extent answers this as with within-subject comparisons.

It is an unexplained observation that although peripheral aromatase activity is inhibited by about 1000 mg AG daily, there is much less than 95% suppression of plasma or urinary oestrogen levels [7]. It has been considered that this may be due to assay "noise" or to an exogenous source of oestrogen such as the diet. Whatever the cause the end result of this is that at present the target level of suppression of oestrogen levels is ill-defined. The approach that has been taken in defining the optimal dose is therefore to select from a series of increasing doses that dose at which no further suppression occurs.

The pharmacokinetics of many drugs vary widely between individuals. Peak circulating levels of 4-OHA after a single oral dose in a relatively homogeneous group of six normal males varied by a factor of about seven [15]. This indicates that although for an individual a single dose may be defined that is the minimum to achieve maximal suppression, this will vary markedly through the population. This is illustrated in Fig. 3 where a comparison is made of the maximum plasma drug levels obtained after the administration of one of four single oral doses of 4-OHA to a group of postmenopausal breast cancer patients. To achieve optimal suppression for the whole population the majority of patients will be overtreated. Thus any side-effects associated with the drug need to be widely separated from the aromatase inhibitory dose (i.e. of the C-type in Fig. 2) if they are not to be expressed in those patients with the highest circulating drug levels and/or the greatest sensitivity to the drug. This latter possibility has to be considered since other biological variables

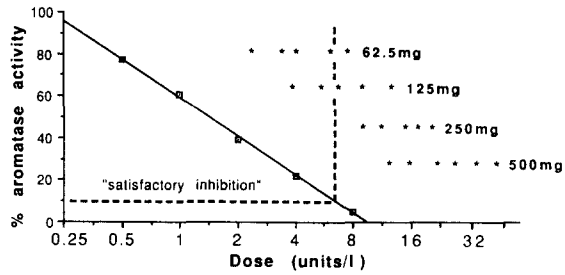

Fig. 3. Peak serum levels of 4-OHA (*) after a single oral dose of 62.5, 125, 250 or 500 mg related to the slope of inhibition of placental aromatase by 4-OHA *in vitro*. Whilst the slope is correct the position of the line is arbitrary as is the designation of 90% as satisfactory inhibition. The diagram illustrates how variable pharmacokinetics can lead to both satisfactory and unsatisfactory inhibition in individual patients at a single dose level.

will lead to an inconsistent response to similar circulating drug concentrations between patients.

If these types of pharmacological study are conducted in patient populations (rather than volunteers) the problem of compliance and concurrent therapies cannot be ignored. The former of these can to some extent be monitored by measurement of blood drug levels. The latter is a matter of good clinical practise.

The statistical approach which has been taken in many studies has been relatively ill-disciplined. The minimum dose to achieve the maximal effect has generally been accepted as that which causes oestrogen suppression which is *not significantly different* from the next highest dose. This will almost certainly lead to acceptance of doses which are not maximally effective for all patients. The more appropriate procedure is to define the criteria by which a dose would be rejected, e.g. if the suppressed levels of oestradiol were n% or x pmol/l higher than that of the highest dose tested. The appropriate mathematical formulae can then be applied to select a number of patients which will have a given probability of detecting such a difference. An alternative or additional approach would be to consider the proportion of patients in which it would be acceptable to achieve such a degree of undertreatment.

Most recently we have added to our measurement of plasma oestrogen levels the direct measurement of peripheral aromatization by radioactive precursor product (i.e. androstenedione/oestrone) injections. These are performed before and during treatment. The analysis is performed on 72 h urine collections which are treated with a new purification and HPLC technique [16]. This technique allows direct measurement of the inhibition of aromatase

activity and its sensitivity allows the measurement of $<5\%$ remnant activity. The procedure is labour intensive and technically demanding, but the information is a very valuable adjunct to the information derived from plasma oestrogen analyses.

PROSTATE CANCER

There is a tenuous rationale for suggesting that aromatase inhibitors might be useful in prostatic cancer despite the very low oestrogen receptor levels in this disease. We have examined both AG and 4-OHA clinically and endocrinologically in patients with metastatic, post-orchiectomy relapsed prostatic cancer [17, 18]. Subjective benefit was derived from both treatments with a notable flare of disease during the early part of treatment with 4-OHA. It is very difficult to ascribe this benefit to aromatase inhibition. The value of aromatase inhibitors in prostatic cancer remains in doubt. It will only be defined by the use of highly-selective aromatase inhibitors, earlier in the disease in randomized, controlled trials.

CONCLUSIONS

The conduct of plasma oestrogen analysis has been a valuable aid to the development of aromatase inhibitors for breast cancer treatment. The most useful information is derived when parallel assays of plasma drug levels are conducted. There are numerous difficulties with the use of plasma oestrogen analyses to define optimal dosage schedules. It is important that such studies are conducted to appropriate, well-defined statistical criteria. The use of radioactive infusion studies is likely to become an essential tool for comparisons between drugs and doses.

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