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AROMATASE INHIBITORS: INTRODUCTION AND PERSPECTIVE

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This special issue of the Journal of Enzyme Inhibition contains a collection of representative papers from most of the investigators currently working on aromatase inhibition.

The field had its beginnings in the early 1970s when we decided to develop specific inhibitors to aromatase (estrogen synthetase).[1] Aromatase is an enzyme complex consisting of a cytochrome P-450 ($P\text{-}450_{AROM}$) hemoprotein and a flavoprotein, NADPH-cytochrome P-450 reductase. Aromatization of C-19 androgens is a key step in the synthesis of estrogens. Thus, we envisaged that aromatase inhibitors could have several important uses. In fact, aromatase inhibitors are proving useful as tools to investigate the role of estrogen in physiological and pathological processes. Some of our initial funding came from the NICHD Contraceptive Development Program as a contract to study innovative antifertility methods. However, I had been interested in the role of estrogens in the growth of breast cancers for a number of years since working at the Christie Hospital in Manchester, England. That aromatase inhibitors might be of therapeutic value seemed a reasonable possibility. There were early indications at that time, that the use of antiestrogens were being effective in breast cancer patients. Also, research on estrogen receptors in breast tumors was beginning to be pursued as a diagnostic procedure to aid in the selection of patients most likely to benefit from endocrine therapy. This further stimulated our interest in developing compounds with a different mode of action from antiestrogens but which might also be of value. All antiestrogens known at that time and until recently were weak or partial agonists as well as antagonists. By using a different approach, we considered we might identify compounds without significant estrogenic activity and our results indicated this to be the case.[2] The possibility existed that aromatase inhibitors might be more effective than or useful alternatives to antiestrogen therapy.

Aromatase inhibitors gained recognition slowly. The work of Dr. Richard Santen and his colleagues utilizing aminoglutethimide (AG) an inhibitor of cytochrome P_{450} steroidogenic enzymes with cortisol replacement therapy, established that inhibition of aromatase was effective in treating breast cancer patients.[3] The early basic studies on steroidal inhibitors and success with AG in patients, gradually generated interest in the field. Clinical studies with 4-hydroxyandrostenedione (4-OHA), the first selective aromatase inhibitor and a mechanism-based inhibitor were difficult to initiate. However, due to the enthusiastic efforts of Dr. R. Charles Coombes, patients finally began to be treated with material which we supplied to him at the Royal Marsden Hospital in Surrey, England in the early 1980s. This work continued at St. George's Hospital in London and early results indicated that patients responded to 4-OHA.[4,5] Development of 4-OHA for clinical use has now been taken up by Ciba-Geigy AG,

Basel (CGP 32349). Recent results suggest that response rates are at least similar to those with tamoxifen.[6,7]

The clinical experience with the antiestrogen tamoxifen is considerable by this time. In 1983, a consensus workshop at NCI evaluated the findings of a large number of investigators and concluded that tamoxifen was statistically more effective in postmenopausal breast cancer patients with estrogen receptor positive tumors than cytotoxic agents.[8] A role for aromatase inhibitors as second line treatment in patients who relapse from tamoxifen was evident. All of these events spurred the recent interest in development of aromatase inhibitors.

The development of new aromatase inhibitors has followed from the two original classes of compounds, steroidal inhibitors such as 4-OHA and non-steroidal or imidazole inhibitors such as AG. There are now a number of potent steroidal inhibitors. These are inhibitors which show type 1 spectral binding and appear to interact with the active-site of the enzyme. Some are mechanism-based inhibitors, such as 4-OHA, 19-acetylenic androstenedione (10-(2-propynyl)estr-4-ene-3,17-dione), 7α-(4′-amino)phenylthio-4-androstene-3,17-dione and a novel steroid androsta-4,6,8,(9)-triene-3,17-dione. These and other 7-substituted steroids as well as a new competitive inhibitor 2,2-dimethyl-4-hydroxy-4-androstene-3,17-dione are discussed in this issue. Mechanism-based inhibitors are compounds which are not intrinsically reactive. Initially, they compete rapidly with the enzyme's natural substrate and subsequently interact with the enzyme, binding to it either very tightly or irreversibly and causing inactivation of the enzyme. Potentially, compounds of this type have long lasting effects *in vivo*, as the continued presence of the drug is not required. This also reduces the chances of toxic side-effects.

Several potent non-steroidal aromatase inhibitors are also described here. These are the so-called "type II" inhibitors and are based upon inhibition of the cytochrome P_{450} due to binding of nitrogen to the hemoprotein component of the enzyme. AG and several imidazole antimycotic agents are inhibitors of this type. Recently, imidazoles such as CGS 16949A (4-(5,6,7,8-tetrahydroimidazo-[1,5a]pyridin-5-yl)-benzo-nitrile hydrochloride) discussed by Bhatnagar *et al.* and by Santen and colleagues, and R 75251 (6-[(4-chlorophenyl) (1*H*-1,2,4-triazol-1-yl)methyl]-1-methyl-1*H*-benzotriazole) from Janssen Pharmaceutical company have been found to be competitive inhibitors of aromatase but have much greater activity compared to AG. Thus, potential for intrinsic lack of specificity is minimized. Also, increased potency would be expected to be associated with fewer side-effects. Patients have experienced a number of troublesome toxicities with AG and with the imidazole antimycotic agents such as ketoconazole.

A further distinction between the effects of the two classes of compounds are apparent because of differences in the physiological regulation of gonadal and peripheral aromatase. Although it is currently believed that a single gene encodes only one species of aromatase in the human,[9] regulation of the enzyme appears to be tissue-specific. Aromatase is regulated by gonadotropins in the gonads but these hormones do not affect peripheral aromatase. Information concerning the regulation of aromatase in other tissues is sparse, except for adipose tissue in which glucocorticoids and growth factors have been shown to be involved.[10] In studies carried out with AG in premenopausal breast cancer patients, it was noted that ovarian production of estradiol could not be consistently suppressed.[11] Initially, estrogen levels are reduced. This results in reflex increases in the gonadotropins LH and FSH. Since these hormones stimulate ovarian steroidogenesis, they override the AG blockade. Some

steroidal inhibitors, however, appear to act by additional mechanisms *in vivo*. For example, independent of its action on aromatase, 4-OHA inhibits gonadotropin secretion in a dose-dependent manner. Thus, low ovarian estradiol production can be maintained. We have demonstrated that 4-OHA inhibits LH and estradiol in the baboon when administered over two or three months.[12] Whether this mechanism will be of advantage in the use of 4-OHA or other steroidal inhibitors in premenopausal patients remains to be determined.

This special issue focuses on the chemistry and biology of several of the more recent compounds as well as on some new inhibitors. The latest data about the compounds which are in clinical trials are reviewed in the papers by Santen *et al.* and Bhatnagar and coworkers.

Aromatase Inhibitors are now reaching the point where they will begin to have an impact on the treatment of postmenopausal estrogen dependent breast cancer. Their utility in other diseases in which estrogens play a role remains for further exploration.

Finally, Dr. Osawa and his colleagues have contributed a fascinating review of naturally occurring aromatase inhibitors which they have identified in cigarettes and some vegetables.

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