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INHIBITION OF OESTROGEN SYNTHESIS IN POSTMENOPAUSAL WOMEN WITH BREAST CANCER

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Summary—The effectiveness in reducing oestrogen exposure, of an aromatase inhibitor, and a sulphatase inhibitor, as measured by *in vivo* studies in breast cancer patients, has been investigated. 4-Hydroxyandrostenedione (4HA) was shown to diminish plasma oestrogen levels, to inhibit peripheral and local aromatization and to cause a concomitant decrease in the activity of DNA-polymerase-α, measured as an indicator of cellular proliferation. The source of oestrone sulphate in breast tissues was examined, and it was shown that the tissue content of this conjugate derived from circulating oestrone, but no evidence could be found for the direct accumulation of conjugate from the plasma. Administration of Danazol was found to cause a fall in plasma oestrone levels, and to diminish the conversion ratio of oestrone sulphate to oestrone in some patients. It also inhibited tissue sulphatase activity. Although it is concluded that this drug is only a weak sulphatase inhibitor, these observations indicate the potential value of developing more efficient sulphatase inhibitors. Enzyme inhibition is now a proven effective treatment for breast cancer and the development of more efficient inhibitors is an important objective.

At some stage in their development, a substantial proportion of breast tumours, if not all, are dependent for their growth and development on the support of oestrogens [1]. The recognition of this phenomenon has led to oestrogen deprivation taking a prime role in the treatment of both early and advanced breast cancer, in many cases with highly successful results. Initially the only means by which this could be achieved was by surgical ablation of oestrogen producing tissues, requiring ovariectomy, and with the recognition of the importance of peripheral oestrogen synthesis from androgens in postmenopausal women, adrenalectomy was also adopted as a therapeutic adjunct. Inhibition of adrenalcortical steroid production can also be achieved by the use of chemical inhibitors of steroid synthesis, leading to the use of such drugs as aminogluthethimide [2]. More recently, attention in this area of therapy has been focused on achieving oestrogen deprivation by the use of aromatase inhibitors, and the compound which has been most widely studied and used for this purpose is 4-hydroxyandrostenedione (4HA) [3]. Although aromatization of androgens is undoubtedly the major, if not the only source of oestrogen synthesis, even after successful inhibition of aromatase activity, there remains within the body a substantial and slowly turning over pool of oestrogen precursor, oestrone sulphate, which through the action of sulphatase, provides a continuing source of oestrone, and hence of oestradiol [4, 5]. Thus, even total inhibition of aromatase activity will still leave body tissues exposed to a further source of oestrogen, and although it is to be assumed that since this source is also dependent ultimately on the action of aromatase, it too will eventually be cleared and not replaced. Nevertheless, the magnitude of the pool size, and the slow turnover, has attracted attention to this significant source of oestrone, and the use of sulphatase inhibitors must be considered as a possible adjunct to treatment with aromatase inhibitors.

Initially, development of successful enzyme inhibitors requires *in vitro* studies with both normal and abnormal tissues, to establish and identify drugs potentially useful for further study and development. Ultimately, however, all such compounds need to be examined by *in vivo* methods and eventually by clinical studies to establish their clinical role. In this paper, we describe the results of our *in vivo* studies on two inhibitors of steroid enzymes: 4HA, currently the most widely used aromatase

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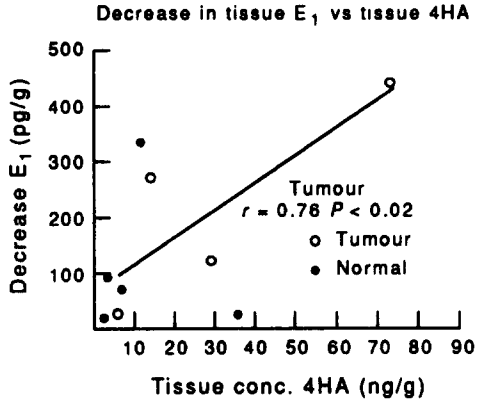

Fig. 1. Correlation between the decrease in breast tumour oestrone concentration and tissue concentration of 4HA.

inhibitor, and Danazol, a drug which has been shown by *in vitro* studies to have significant activity as an inhibitor of steroid sulphatase activity [6, 7].

The general approach which we have used has been described in detail in previous papers [8–10]. We have combined the measurement of steroid concentrations in blood and tissues with the use of an isotopic infusion technique to permit kinetic measurements to be made, and to enable us to discern more clearly the metabolic pathways involved which are influenced by the administration of these drugs.

INHIBITION OF AROMATASE

The effect of administering 4HA to a group of 8 postmenopausal women with advanced breast cancer was studied. These patients were given two doses of 500 mg of 4HA at an interval of 12 days, and were investigated immediately prior to, and 36 h after the second treatment with the drug. Samples of normal and tumour tissue were examined. The tissue concentration of oestrone fell from a mean pretreatment level of 140 to 51 pg/g after treatment in the normal tissue, and from 321 to 112 pg/g in the tumour tissue [11]. Mean plasma concentrations of oestrone fell from 27 to 14 pg/ml, and of oestradiol from 10 to 4 pg/ml [12]. The concentration of 4HA in plasma was 28 ng/ml, and was 14 and 33 ng/g in normal and tumour tissue, respectively [11]. Concentrations in plasma and normal tissue were correlated. Overall, no significant correlation was detected between the decrease in tissue oestrone concentration and concentrations of 4HA ($r = 0.58$, NS). For the tumour samples, however, the correlation was significant ($r = 0.76$, $P < 0.02$) (Fig. 1). It might be anticipated that the changes in concentration of oestrone in plasma and tissue might be correlated but this was not always the case, and so plasma concentrations can not be used with confidence to predict tissue concentrations. The biological effect of the diminished oestrogen levels was investigated by measuring the activity of DNA-polymerase-α in the tumour tissues before and after treatment. In three of the four samples measured, there was a considerable decrease ($78 \pm 4\%$) of the concentration of oestrone, and in these three patients DNA-polymerase decreased by $64 \pm 16\%$. In the other patient there was only a small fall in oestrone concentration and no change in DNA-polymerase-α.

In our earlier studies we also examined the effect of 4HA on the aromatase activity of the tumours and compared this with the changes in DNA-polymerase activity [13]. In five out of the six patients studied, there was a 50% or greater reduction in tissue aromatase activity measured *in vitro*, and polymerase activity was also diminished in general. Surprisingly though, one patient showed an increase in both these parameters. Using the isotopic infusion technique, the *in situ* synthesis of oestrone accounted for the major part of the oestrone detected, whereas after treatment, *in situ* activity could only be detected in one patient. In addition, the peripheral aromatization of androstenedione to oestrone was measured, and after treatment with 4HA, conversion was almost completely inhibited [12].

From these investigations we can draw a number of conclusions. After the administration of the inhibitor, 4HA, it is possible to detect significant concentrations of the drug in both plasma and tissues. The concentrations in normal tissue were lower than in tumour tissue and although the concentration of drug in plasma correlated with that found in the normal tissue, it did not do so with that in the tumour. However, it is difficult to attach too much significance to this finding since it was not entirely certain that steady state had yet been achieved. When the effect of 4HA on aromatization is examined, we found that the drug caused highly efficient inhibition of peripheral aromatization, which became virtually undetectable. This effectiveness was reflected in the finding that the *in situ* production of oestrone was strongly suppressed in tumours in which prior to treatment a large proportion of oestrone was being synthesized within the tumour. In addition, aromatization measured *in vivo* was

also markedly depressed. The fact that these changes in steroid biosynthetic activity were also accompanied by a general fall in the activity of DNA-polymerase activity, suggests that the changes induced in the oestrogen concentrations were biologically effective in altering tumour growth rates. This conclusion is also supported by the histological examination of the tissues, which showed evidence of nuclear vacuolation induced by the drug.

The administration of 4HA caused a significant fall in the plasma concentration of both oestrone and oestradiol, in agreement with the observations of other workers [15, 16]. Nevertheless, although these concentrations fell, there was still present in plasma substantial amounts of both oestrogens, even after several days of therapy. Thus, given the difficulty of measuring small amounts of oestrogen, it appears that there is still a significant source of oestrogen present even when effective inhibition of aromatase is occurring.

INHIBITION OF SULPHATASE

This alternative source seems likely to be oestrone sulphate [17]. There are very substantial amounts of this steroid present in both plasma and tissues, and the compound is not only widely distributed, but also has a slow metabolic clearance rate [18]. Through the action of sulphatase, it provides a continuing source of oestrone, even when oestrogen production has been blocked. These considerations have led to the concept that inhibition of sulphatase activity in conjunction with inhibition of aromatase, might also prove to be a useful therapeutic approach, and this idea is supported by a body of evidence from both *in vivo* and *in vitro* experiments, showing that oestrone sulphate provides a biologically significant source of oestrone [4, 5].

In contrast to the substantial advances in the development of aromatase inhibitors, there has been relatively little progress in this area, and to date, no clinically useful sulphatase inhibitors have been reported.

Some years ago, Carlstrom *et al.* [7] reported that Danazol, a synthetic derivative of testosterone, was partially active as a sulphatase inhibitor *in vitro*, and after administration *in vivo*, levels of dehydroepiandrosterone decreased, and the levels of its sulphate increased, thus suggesting that the drug was causing inhibition of sulphatase activity. We have therefore studied the effect of this drug in patients with advanced breast cancer, and we have also explored some relevant aspects of the metabolism of this steroid conjugate.

Several studies have shown that oestrone sulphate is capable of stimulating tumour growth *in vitro* and *in vivo*, but the experimental evidence does not distinguish between a direct effect of the steroid conjugate, and the indirect mechanism, by which oestrone sulphate undergoes preliminary hydrolysis to oestrone [19, 20]. Although oestrone sulphate is present in human breast tumours, it is not clear if it is taken up as such by the tumour cells, or is produced *in situ* from oestrone by the action of sulphokinase. We have therefore examined this possibility using isotopic tracer techniques.

After the infusion of [^{3}H]oestrone sulphate to a group of women with breast cancer, [^{3}H]oestrone sulphate was present in the tumour tissue removed at subsequent surgery. The tissue–plasma ratios were < 1 and there was no difference between normal tissue and tumour tissue. After treatment with Danazol, no change in tissue–plasma ratio could be detected. Similar tissue–plasma ratios in both normal and tumour tissue were found after the infusion of [^{3}H]oestrone [21].

In order to examine the uptake of oestrone sulphate directly by breast tissues, in the next series of experiments, oestrone[^{35}S]sulphate was infused, and the tissue removed at surgery was examined. In these studies it was not possible to detect any significant radioactivity in the tissues [23].

The conversion ratios of oestrone sulphate to oestrone were measured, before and after treatment with Danazol. In 2 of the 3 subjects studied, the conversion ratio fell by 40%, but no change was detected in the third. No overall significant change could be found in the metabolic clearance rate of oestrone sulphate. After treatment for 2 weeks, plasma oestrone concentrations decreased by 30%, and the sulphatase activity measured in normal breast tissue also decreased.

These studies thus demonstrate that after infusion of [^{3}H]oestrone, it is possible to detect [^{3}H]oestrone sulphate in breast tissues. However, the observation that there was no apparent uptake of oestrone sulphate labelled with ^{35}S appears to eliminate the possibility of direct uptake of this conjugate, as might be expected from its strongly polar character. The likely mechanism would thus seem to be by hydrolysis

and then subsequent resynthesis of the conjugate in the tissue.

Our studies of Danazol as a sulphatase inhibitor have shown that this compound will produce some diminution in plasma oestrone levels and will also in some subjects cause a fall in the conversion ratio of oestrone sulphate to oestrone. A diminution in tissue sulphatase activity also occurred. However these changes were not seen consistently in the group of patients studied, and the extent to which sulphatase inhibition occurred suggests that Danazol is at best only a relatively weak sulphatase inhibitor.

CONCLUSIONS

The use of enzyme inhibitors in the treatment of breast cancer now has an established role, having been demonstrated to be of proven clinical effectiveness. To date, aromatase inhibitors have been the most successful drugs in this field, and 4HA was found to reduce plasma oestrogen levels and to inhibit both peripheral and breast tissue aromatase activity. However, in the studies reported so far it has not been possible to obtain complete elimination of circulating oestrogen and it seems likely that other sources of oestrogen are still making a significant contribution. Since oestrone sulphate is a major precursor, the development of sulphatase inhibitors would clearly be a useful step towards the goal of rapid and complete oestrogen deprivation in breast cancer patients.

Further studies of steroid metabolism, and *in vitro* studies of steroid enzyme inhibitors are needed, followed ultimately by *in vivo* work and then clinical trials to improve and enlarge the scope of what is already an effective additional approach to the clinical treatment of human breast cancer.

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