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EFFECT OF TREATMENT WITH DANAZOL ON IN VITRO AND IN VIVO OESTROGEN SULPHATASE (EST) ACTIVITY IN BREAST CANCER.

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Conversion of oestrone sulphate (E_1S) to oestrone (E_1) by EST may make an important contribution to the oestrogen content of malignant (M) and normal (N) breast tissues (BT). To investigate this possibility, we have examined: (i) the in vivo uptake of ^{3}H-E_1S by MBT and NBT and the conversion of E_1S to E_1 before and after treatment (400 mg bd for 2 weeks) with Danazol, which may inhibit EST activity; (ii) EST activity in MBT and NBT before and after treatment with Danazol and (iii) EST activity in MCF-7 and MDA-MB-231 breast cancer cells. Tissue:plasma ratios after infusion of ^{3}H-E_1S were 0.18±0.09 for MBT and 0.16±0.12 for NBT. The conversion ratio for the conversion of E_1S to E_1 decreased from 3.4±1.2% (mean±S.D.) to 2.2±0.9% ($p<0.02$) after treatment with Danazol. EST activity in MBT was significantly ($p<0.01$) higher than in NBT but was not reduced in all samples of MBT examined after treatment. EST activity in MDA-MB-231 cells was significantly higher ($p<0.01$) than in MCF-7 cells and EST activity in these cells was inhibited by Danazol (10uM). The higher EST activity found in MBT than NBT suggests that conversion of E_1S to E_1 may contribute to the formation of biologically active oestrogens in breast tissues and that Danazol may act to reduce conversion of E_1S to E_1.

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AROMATASE INHIBITORS AND HEPATIC SULPHATASE

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Aminoglutethimide (AG) increases oestrone sulphate (ES) metabolism. One of several possibilities for a mechanism is induction of E sulphatase. We have examined the effect of AG (5-100mg/kg/day, 4 days) in male and female rats and of AG, pyridoglutethimide (PG), the pyrolidinediones WSP1 and 3, 4-OH-androstenedione (OHA), danazol (D) and ethisterone (E) added in vitro to liver microsomes from female rats on hepatic microsomal sulphatase. Sulphatase was assayed (Milsom et al. 1972, Biochem. J. 128, 331) with K^+-4-acetylphenylsulphate as substrate. Hepatic enzyme induction was confirmed by measuring 4-nitroanisole O-demethylation, AG being compared with phenobarbitone (PB 40mg/kg/day). PB and AG (the latter dose-dependently) induced O-demethylation that was more marked in female than male rats. Neither drug influenced hepatic sulphatase activity. AG, PG, WSP1 and 3 and E (15-500mM), added to microsomes, did not affect sulphatase activity. However, OHA (50-500μM) and D (15.6-2000μM) produced a dose-dependent decrease in sulphatase activity (max inhibition 63 and 72% respectively). The results indicate that AG does not affect sulphatase and lend support to the suggestion (Lonning et al. 1988, Drugs 35, 685) that AG enhances ES metabolism by induction of 16α hydroxylase. However, for OHA, sulphatase inhibition may contribute to its oestrogen lowering action.

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AROMATASE CONTENT IN DMBA-INDUCED MAMMARY TUMORS

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The DMBA (7,12-dimethyl-benz(a)anthracene)-induced mammary carcinoma represents a useful animal model of hormone dependent breast cancer. This model has been extensively used in the development of novel drugs for the treatment of breast cancer in postmenopausal women. Previously, the size of the tumors has been used as a parameter to follow their development after DMBA induction. This study describes the measurement of the aromatase content in rat mammary tumors of different weights following induction with DMBA.

50-day old female Sprague-Dawley rats were induced with a single oral dose of 15mg DMBA. Tumor growth was followed in all rats. Groups of rats were weighed and sacrificed every week after 8 weeks of DMBA induction period, for upto 16 weeks. At each week approximately 15 tumors were removed (from the smallest to the largest available). Only progressively growing tumors identified during weeks 5-8 after DMBA administration were taken. Tumors were frozen and stored at -20° C. Aromatase activity was measured by tritiated water assay using [1β-3H]-androstenedione as a substrate. A known inhibitor of aromatase, CGS 16949A was used as a reference drug to validate the assay.

Aromatase activity was also determined in the sub-cellular fractions of a DMBA-induced tumor inorder to establish maximum quantification of the aromatase content. The 1000g supernatant was subsequently used for the measurement of aromatase.

The weights of the tumors analysed ranged from 1 to 47 g and had an average protein content of 60.9 mg/g tumor. The aromatase activity and the total amount of aromatase ranged from 7 to 43 fmol/h/mg protein and from 0.64 to 77 pmol/h/tumor, respectively.

Using the tritiated water assay method, all tumors had aromatase, the overall specific activity range being 4-5 fold. The total amount of aromatase was roughly in proportion to tumor weight but was independent of the time period after induction with DMBA.

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PHASE I STUDY IN HEALTHY MALE VOLUNTEERS WITH THE NON-STEROIDAL AROMATASE INHIBITOR CGS 20267

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The new, non-steroidal aromatase inhibitor, CGS 20267, has been pre-clinically profiled as a potent, selective and efficacious inhibitor of estrogen biosynthesis in vitro and in vivo.
In an open, single dose Phase I study, 18 healthy male volunteers received oral doses of CGS 20267 (3 at each dose) ranging from 0.002 to 3 mg or placebo. Serum concentrations of estradiol (E2) and estrone (E1) were measured at the start of treatment (0 hr) and 24 hours (24 hr) later. E2 and E1 were measured using highly specific and sensitive radioimmunoassays. Results (Mean±S.D.) are presented below:

Dose of CGS 20267	Serum Estradiol pg/ml		Serum Estrone pg/ml	
mg	0 hr	24 hr	0 hr	24 hr
0.002	22.5±7.9	13.9±5.4	21.5±9.6	11.9±3.9
0.250	21.2±3.2	5.3±0.6	18.4±2.0	2.7±0.2
3.0	36.7±7.6	3.2±1.0	24.4±0.4	3.0±0.5

E2 and E1 levels in these subjects, 24 hours after a single dose of CGS 20267, were reduced by about 50% of baseline with the lowest dose of 0.002 mg and by about 90% of baseline with the highest dose of 3 mg. Maximal suppression, however, was already achieved with the dose of 0.25 mg. With the two highest doses, suppression of estrogen levels was maintained for 2-3 days. All doses of CGS 20267 were very well tolerated.
Thus, CGS 20267 has been shown to be a very potent, long acting and very well tolerated aromatase inhibitor in this study.