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DIRECT INHIBITION OF HUMAN BREAST CANCER CELLS BY AN LHRH AGONIST

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Agonist analogues of luteinizing hormone-releasing hormone (LHRH) have been used successfuly to treat premenopausal women with advanced breast cancer. The effects have been assumed to result from the pertubation of the pituitary-ovarian axis which reduces circulating oestrogen to castrate levels. However, responses have been reported in postmenopausal women in whom LHRH agonist treatment does not affect plasma oestrogen. The aim of the present study was to determine whether LHRH analogues can have direct effects on breast cancer cells.

Cells were cultured in Dulbecco's MEM media supplemented with 10% foetal calf serum. Hormone additions were made on Day 0 and cells were counted after 1,2,3 and 4 days of culture, media being changed daily. Triplicate systems were set up for each system at each time point.

Concentrations of LHRH agonist, buserelin, in excess of 10-9M consistently suppressed growth in monolayer of the oestrogen sensitive cell line MCF 7 over the 4 day culture period. The inhibitory effects were dose-related such that levels of buserelin greater than 10-7M produced a progressive decline in cell numbers. Native LHRH was also inhibitory but high concentrations (>10-6M) were required; the 3-10 fragment of LHRH had no measurable effects. The suppressive effects of LHRH agonist were completely blocked by addition of an LHRH antagonist or by inclusion of insulin in the culture media. In contrast, oestradiol partially reversed the effects of LHRH agonist.

These actions of LHRH analogues were at least partly specific to MCF-7 cells, effects being minimal on the growth of the MDA-MB-231 and T-47D human breast cancer cell lines and the HBL-100 human "normal" breast line.

Specific binding sites for LHRH agonist have also been demonstrated in MCF-7 cells although their role in mediating effects on cellular events is questionable in view of their relatively low affinity (Kd ~ 10-9) and their presence in other breast cancer cells such as MDA-MB-231 which have not been shown to be sensitive to LHRH agonists.

It is concluded that LHRH analogues have the potential to inhibit directly certain breast cancer cells by means of a specific recognition mechanism. There is circumstantial evidence that the effects may be anti-steroidal as has been reported for LHRH agonists in other tissues. These observations have important implications for the use of LHRH analogues in the treatment of breast cancer.

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DIRECT EFFECTS OF LHRH ANALOGS ON TUMOR CELLS

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In addition to endocrine effects in vivo neuropeptides such as luteinizing hormone-releasing hormone (LHRH) may also act by autocrine or paracrine mechanisms. We have studied the effects of a potent agonist of LHRH, buserelin (BUS), in combination with other endocrine measures, on the proliferation of MCF-7 human breast cancer cells. To study the effects of BUS, oestradiol (E_2) concentrations comparable to the plasma values in medically castrated (by LHRH agonists) patients, and slightly above this level, were used. In medium supplemented with 10% steroid-depleted male human serum, concentrations of 30-80 pM E_2 already resulted in stimulation of the growth of the cultures of more than 500% as measured by protein and DNA content at day 7. This induced growth could be inhibited by BUS in a dose-dependent manner resulting in 96% (at 30 pM E_2) and 71% (at 80 pM E_2) inhibition with 0.8 µM BUS. In cultures in the presence of E_2, BUS did not change the patterns of secreted proteins when analyzed by SDS-PAGE followed by fluorography. No effects of BUS on the levels of cytoplasmic and nuclear oestradiol receptor could be observed whereas the E_2-induced synthesis of the progesterone receptor was inhibited. In addition, while both tamoxifen (TAM) and BUS, when administered separately, could block the E_2-stimulated growth, a combination of TAM and BUS was less favourable in this respect. In a clinical study however tumor regression did occur after administration of both drugs. In conclusion, these results suggest that precise balances between E_2, BUS and/or TAM are required to obtain maximal inhibitory effects, at the cellular level, on the growth of breast cancer cells. Clinical importance of these observations has yet to be evaluated. (This study was supported by grant RRTI 83-5 of the Netherlands Cancer Foundation).

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MECHANISMS OF GROWTH REGULATION OF HUMAN BREAST CANCER.

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The mechanisms by which estrogens and antiestrogens regulate the growth of human breast cancer have not been completely elucidated. Substantial amounts of information, often conflicting, have suggested on the one hand that estrogens exert no direct growth regulatory effects on breast cancer(BC)(1), while other work has suggested that estrogens directly regulate gene expression in human BC (2). In this presentation data will be reviewed supporting 4 hypotheses:

I. In human BC estrogens directly alter transcription of specific genes in BC and these gene products are involved in the growth response.

II. In hormone dependent human BC cells, estrogens increase and antiestrogens decrease the secretion of potent autocrine and paracrine growth factors. These growth factors are directly responsible for tumor growth and progression. They include insulin-like growth factor I, transforming growth factor α, platelet derived growth factor and a novel epithelial growth factor.

III. Hormone independence is conferred by the ability of BC cells to constituitively secrete a similar spectrum of autocrine and paracrine growth factors.

IV. The effects of antiestrogen and other growth inhibitors are induced by two mechanisms. First, by inhibition of stimulatory autocrine and paracrine growth factors and secondly, by induction of intracellular and extracellular factors with inhibitory activity. Growth inhibitory substances produced by hormone dependent cells can limit growth of hormone independent cells.