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LHRH BINDING SITES IN HUMAN PRIMARY BREAST TUMOURS; P Mullen, TA Bramley† WR Miller: I.C.R.F., Medical Oncology Unit & University Dept of Obs & Gyn*, Edinburgh.

LHRH agonists have been used to treat women with metastatic breast cancer. Whilst benefits in premenopausal women appear to be mediated by supression of the pituitary gonadal axis, studies of breast cancer cell lines suggest that LHRH agonists may be capable of direct inhibitory effects, implying the existance of specific tumour receptor sites. The presence of LHRH binding sites in human primary breast tumours and cell lines has therefore been investigated using iodinated LHRH and analogues.

Incubation conditions were optimised for temperature, tubes, pH, Ca^{2+}, Mg^{2+}, EDTA and assay buffer. Both glass and plastic tubes were used. Several ligands were studied, including salmon, chicken, lamprey and mammalian GnRH, along with GnRH analogues. Ligand degradation was monitored throughout. Despite these modifications, no specific high affinity LHRH binding sites could be detected although their presence was found in human placenta and rat pituitary.

In contrast to other studies (Eidne et al, 1985) it has not therefore been possible to provide evidence that action of LHRH agonists on breast cancers is mediated through specific receptor sites.

(Eidne et al: Science; 229, 989-991)

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DIRECT INHIBITION OF POLYOMAVIRUS INDUCED PROGRESSIVE MAMMARY TUMORS IN ATHYMIC NUDE MICE BY THE LONG-TERM ADMINISTRATION OF A GONADOTROPIN RELEASING HORMONE AGONIST.

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Clinical reports on remissions in postmenopausal breast cancer patients, and *in vitro* results on growth inhibitions of breast cancer cell lines point out the direct antitumoral potency of gonadotropin-releasing hormone (GnRH) agonists. To define which steps of the mammary carcinogenesis the GnRH agonist direct action could target, the efficiency of the sustained release form of D-Trp^6 GnRH (Decapeptyl, IPSEN Biotech) was tested on Polyomavirus (Py)-induced mammary carcinogenesis in BALB/c female athymic nude mice. Because it displays both ovarian hormone-dependent (tumor promotion in ductal cells) or -independent (tumor growth) stages, this model is of particular interest for such a study. Tumor growth was unaltered by long-term treatments (5 mg/Kg) starting prior to Py injections. The tumor appearance kinetics were similarly affected in normal mice with estradiol and prolactin decreased seric levels, and in estrogen-supplemented ovariectomized mice without inhibition of estradiol or prolactin seric levels. In addition to pituitary-mediated effects, this GnRH agonist thus exerts direct antitumoral effects on Py-transformed, hormone-sensitive mammary cell populations, likely at the beginning of the tumor progression. Our data raise the question of whether GnRH agonists directly affect the growth factor secretion in breast tumors, with ongoing consequences for their uses in clinical situations. This experimental model is shown to be useful in studying the antitumoral potencies of actual or future GnRH analogs, and in investigating the basic mechanisms of the mammary carcinogenesis.

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EFFECT OF A NEW LHRH ANTAGONIST ON DMBA-INDUCED MAMMARY TUMOURS

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The immediate onset of gonadal steroid suppression after LHRH antagonists is a clinical advantage in oncology and reproductive research. A novel glycosylated peptide (1), [AcD-Nal(2),D-pCl-Phe,D-Trp,Ser,Tyr,D-Ser(Rha), Leu, Arg, Pro, AzGly-NH2]LHRH, was evaluated for its biological potential in experimental tumours, and pharmacokinetics. Methods: female rats were treated sc and with controlled release preparations of the antagonist and the LHRH agonist buserelin in poly-lactide/ glycolide. Pituitary hormones concentrations of agonist and antagonist were measured by specific RIA. Tumours were induced by DMBA 3 weeks before treatment. Results: Infusion of antagonist 60-120 ug sc per day reduced uterine weight in a similar manner as castration. The controlled release formulation (3.6 mg antagonist per rat q. 2 weeks) suppressed tumour development for 6 weeks and was nearly as effective as ovariectomy. Tumour growth was also delayed for 6 weeks after a single dose of 3.6 mg buserelin microparticles. The organ distribution of 125-I-labelled antagonist showed long-lasting pituitary accumulation, and a terminal serum half-life of 60 h, and a prolonged urinary and biliary excretion. Receptor affinity for rat pituitary membranes was 10x higher than buserelin. No symptoms of histamine release were found in dogs after 0.5 mg/kg iv, and 0.5-1 mg/kg iv/sc were well tolerated by mice, rats, guinea pigs and rabbits. Conclusions: This LHRH antagonist is highly effective and well tolerated, its long duration of action indicates an important clinical potential.

(1) König W, Sandow J, Jerabek-Sandow G, Kolar C 1989; In Peptides 1988: Proc. 20th European Peptide Symposium (eds. G Jung, E Bayer), Walter de Gruyter Verlag Berlin, pp. 334-336.

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D 20453 - A POTENT ANTAGONIST OF LH-RH IN THE TREATMENT OF DMBA INDUCED MAMMARY CARCINOMA

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The decapeptide D 20453 is a new and highly potent LH-RH antagonist originally synthesized by BAJUSZ and co-workers (1). D 20453 is free of anaphylactoid effects. The suppression of LH and FSH levels was confirmed in several species. The activity of D 20453 in estrogen-dependent tumors was assessed in rats bearing DMBA induced mammary carcinomas. Within the range of 0.316 to 316 µg/kg/day a dose-dependent tumor growth inhibition was found. Histologically apoptosis was a predominant feature associated with tumor shrinkage. The therapeutic efficacy was comparable to that of ovariectomy. Treatment of ovariectomized tumor-bearing rats, which were given replacement therapy with β-estradiol, did not result in tumor regression, indicating that D 20453 acts predominantly via the pituitary-gonadal axis. Initial clinical observations confirmed the safety and efficacy of a single dose in patients with gonadal dysfunction (2). Phase II studies will be performed in patients with prostatic cancer.

(1) Bajusz; S. et al. Int J Peptide Protein Res 32:425-435 (1988)
(2) Gonzáles-Bárcena, D. et al. Annual Meeting of the Endocrine Society, 1989

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