Inhibition of growth of MCF-7 MIII human breast carcinoma in nude mice by treatment with agonists or antagonists of LH-RH

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Summary

Human breast carcinoma (MCF-7 MIII), which exhibits an estrogen-independent but estrogen-responsive phenotype, was xenografted in 8-9-week-old intact female athymic nude mice without estrogen supplementation. In this model, we investigated inhibitory effects of the modern luteinizing hormone-releasing hormone (LH-RH) antagonist SB-75 and the agonist D-Trp6-LH-RH. The analogs were administered in the form of sustained delivery systems (microcapsules and microgranules). In the first experiment, treatment lasted 10 weeks. After 9 weeks of treatment, a significant inhibition of tumor volume was first found only in the group treated with SB-75, but the final tumor volume was significantly suppressed both by D-Trp6-LH-RH and SB-75. In the second experiment, treatment was started 70 days after tumor transplantation and was continued for 6 weeks. Chronic treatment with SB-75 or D-Trp6-LH-RH appeared to completely arrest tumor growth as measured by tumor volume, percentage change in tumor volume, and tumor weight. Serum estradiol was suppressed to undetectable levels and LH levels were also diminished. Histologically, the regressive changes in the treated tumors were due to the enhancement of apoptosis (programmed cell death) of tumor cells. Membrane receptor assays showed that LH-RH binding sites were down-regulated in tumor cells after treatment with SB-75 or D-Trp6-LH-RH.

The results indicate that the antagonist SB-75, released from sustained delivery systems, can inhibit the growth of MCF-7 MIII tumors as effectively as the agonist D-Trp6-LH-RH, but more rapidly. In view of its immediate blockade of the pituitary-gonadal axis and the absence of side effects, the LH-RH antagonist SB-75 might be considered as a possible new hormonal agent for the treatment of breast cancer.

Introduction

Among all newly diagnosed cancers in women in the United States, the incidence of breast cancer is highest and accounts for about 30% of all malignancies [1]. Beatson showed in 1896 [2]...
that surgical oophorectomy caused tumor regression in some premenopausal patients with breast cancer. Since then a variety of hormonal therapies for breast cancer have been developed as alternatives to oophorectomy [3]. In recent years tamoxifen, a non-steroidal antiestrogen, has become the endocrine therapy of choice because of its efficacy and low toxicity, but it does not completely antagonize the effects of ovarian estrogens [3]. Surgical oophorectomy is invasive, irreversible and unacceptable to some patients, although it results in rapid reduction of estrogen levels. New approaches, which cause sufficient estrogen deprivation immediately, should be explored to improve therapy.

Since the isolation and structural elucidation of hypothalamic luteinizing hormone-releasing hormone (LH-RH), more than 3000 LH-RH analogs have been synthesized with a view to their potential medical applications [4,5]. Chronic administration of LH-RH analogs leads to inhibition of the pituitary-gonadal axis. This chemical castration is reversible and provides a marked suppression of ovarian estrogen production [6]. The efficacy of LH-RH analogs for treatment of endocrine-dependent cancer was first shown in the dimethylbenzanthracene-induced rat mammary carcinoma model [7]. The results of clinical trials with several LH-RH agonists, including D-Trp6-LH-RH, in women with advanced breast cancer are encouraging [8-15]. The overall response rates range between 30 and 50% in premenopausal women [8-12], and are approximately 10% in postmenopausal women [11,13-15].

While repeated administration of LH-RH agonists is required to inhibit LH and FSH release and reduce the levels of sex steroids, similar effects can be obtained immediately with a single dose of LH-RH antagonists [4,5]. LH-RH antagonists were synthesized initially for contraception [4]. However, progress in the development and clinical application of LH-RH antagonists has been slow. High dose requirements due to the low potency of earlier compounds, along with side effects related to histamine release of analogs with D-arginine in position 6, delayed their clinical use [4,5].

Modern LH-RH antagonists, free of edematogenic and anaphylactoid reactions, containing neutral hydrophilic D-ureidoalkyl amino acids such as D-citrulline and D-homocitrulline at position 6, were recently synthesized in our laboratory and tested in vivo and in vitro [16,17]. Among these analogs, [Ac-D-Nal(2)1,D-Phe(4Cl)2,D-Pal(3)3,D-Cit6,D-Ala10]-LH-RH (SB-75) was shown to be one of the most powerful antagonists in blocking ovulation and inhibiting LH-levels in rats. The use of LH-RH antagonists in cancer therapy would prevent the temporary clinical "flare" of disease that occurs initially in response to LH-RH agonists.

Sustained delivery systems such as microcapsules and microgranules have made treatment more convenient and more efficacious for delivering therapeutic doses of analogs than multiple daily administrations [18]. Such formulations can be injected at monthly intervals. We have demonstrated the efficacy of microcapsules of the LH-RH agonist D-Trp6-LH-RH experimentally [18,19] and clinically [20]. The sustained delivery systems of SB-75 have been proven effective in experimental cancer models [19,21].

The human breast cancer cell line MCF-7 is fully estrogen-dependent for growth in ovariectomized nude mice [22]. The MCF-7 MIII subline was isolated from MCF-7 tumors proliferating in ovariectomized athymic nude mice and was further selected in vitro under estrogen-deprived conditions [23]. While MIII cells are fully estrogen-independent in vitro, MIII tumor growth in nude mice is stimulated by estrogen supplementation. These cells also retain some sensitivity to the inhibitory effects of anti-estrogens in vitro [24], and appear to have acquired an intermediate estrogen-independent but estrogen-responsive phenotype [23,24]. The MIII tumor xenografts in nude mice provide a novel model to investigate the efficacy of new hormonal therapies for human breast cancer in vivo.

This paper reports the inhibitory effects of sustained delivery systems of the LH-RH antagonist SB-75 or the agonist D-Trp6-LH-RH on the growth of human breast cancer (MCF-7 MIII)