Report

Progestin inhibition of estrogen-dependent proliferation in ZR-75-1 human breast cancer cells: Antagonism by insulin

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Abstract

The effect of R5020 [17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione], a synthetic progestin, was studied in the hormone-responsive ZR-75-1 human breast cancer cell line. Following a 12-day incubation with increasing concentrations of R5020, the mitogenic effect of 17β-estradiol (E2, 1 nM) was partially (60-80%) antagonized by the progestin, with a half-maximal effective concentration measured at about 30 pM. This effect of R5020 was completely reversed by the addition of physiological concentrations of bovine insulin, as well as by the potent antiprogestin RU486 [17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(1-propynyl)-4,9-estradien-3-one], but not by the antiandrogen hydroxyflutamide (α,α,α-trifluoro-2-methyl-4'-nitro-m-lactotoluidide). Moreover, the effect of R5020 required the presence of estrogens, thus further indicating a progesterone receptor (PgR)-mediated effect. Low (< 100 nM) concentrations of R5020 increased the specific binding of [125I]-insulin up to 2- to 2.5-fold in intact ZR-75-1 cells, an effect which was reversed by RU486. The effect was rapid, being nearly maximal after 24 h of incubation with R5020. The PgR-mediated effect of R5020 on cell proliferation was abolished by the addition of a pure steroidal antiestrogen. The present results suggest a physiological role for progestins in increasing the responsiveness to insulin, which could, in turn, reverse the antiproliferative effect of progestins on estrogen action and thus decrease the efficacy of progestins in the treatment of breast cancer.

Introduction

Endocrine therapy of advanced breast cancer with high doses of synthetic progestins such as medroxyprogesterone acetate and megestrol acetate has received much attention as an alternative or a follow-up to antiestrogen (tamoxifen) treatment [1, 2]. The functional significance of PgR in the endocrine control of human breast cancer cell growth is controversial. Although PgR expression is clearly under estrogenic control in most experimental models of hormone-responsive human breast cancers [3-5], there is no strict correlation between PgR induction and mitogenic response to estrogens. For instance, the estrogen-responsive MDA-MB-134 and T47D (clone 11) human breast cancer cell lines have estrogen-insensitive, constitutively low [6] and high [7] PgR contents, respectively. Another example is the T47Dco variant cell line, which has extraordinarily high PgR intracellular levels with extremely low, if any ER content [8, 9]. Progestins such as R5020 strongly decrease the mitogenic ef-
fect of estradiol (E$_2$) in T47D (clone 11) cells while having very little effect at physiologically relevant concentrations in the absence of estrogens [7, 10]. These findings have been challenged by the recent report of a mitogenic action of R5020 in T47D cells grown in phenol red- and estrogen-free medium [11].

The present study is aimed at investigating in detail the PgR-specific effects of progestins in a highly estrogen-sensitive model of hormone-responsive human breast cancer, the ZR-75-1 cell line [12]. These cells, in addition to showing a pronounced mitogenic response to estrogens [5, 13] are strongly growth-inhibited by physiological concentrations of androgens [14] and glucocorticoids ([15]; Hatton A-C and Labrie F, unpublished results), the effects of which are additive to the anti-proliferative effect of antiestrogens. It was thus of interest to further document the specificity of the role of progestins in the growth regulation of these cells as well as their interaction with the other steroid receptor systems. Because of its high specificity for PgR binding and its high progestational potency, the synthetic progestin R5020 was chosen for these studies. The effect of R5020 on the binding capacity of radiolabeled insulin was also investigated as a possible mechanism for the specific reversal of PgR-mediated inhibition of ZR-75-1 cell growth by insulin.

Materials and methods

Chemicals

[17α-methyl-3H]R5020 (sp.act. 87 Ci/mmol) and unlabelled R5020, as well as receptor-grade [125I-Tyr$^A_1$]-labelled porcine insulin (sp. act. 1300 Ci/mmol), were purchased from New England Nuclear (Lachine, Quebec, Canada). Dexamethasone (DEX), bovine and porcine insulins, and all media and supplements for cell culture were obtained from Sigma, except for fetal bovine serum which was purchased from Flow Laboratories. Estradiol (E$_2$) and 5α-dihydrotestosterone (DHT) were from Steraloids (Pawling, NY).

The non-steroidal antiandrogen hydroxyfluta-mide (OHF) [16, 17] was generously provided by Dr. J. Nagabushin and R. Neri (Schering Corporation, Kenilworth, NJ). The multivalent steroid antagonist RU486 [18] was a gift from Roussel-UCLAF (Romainville, France). The new steroidal antiestrogen ICI164384 [19] was kindly provided by Dr. A.E. Wakeling (Imperial Chemical Industries, Macclesfield, England).

Cell culture and growth experiments

The ZR-75-1 human breast cancer cells were obtained from the American Type Culture Collection at their 83rd passage. Stock cells were routinely cultured in phenol red-free [20, 21] RPMI 1640 medium supplemented with 10 nM E$_2$, 1 mM sodium pyruvate, 2 mM L-glutamine, 15 mM HEPES, 100 IU penicillin per ml, 100 μg streptomycin sulfate per ml, and 10% (v/v) fetal bovine serum as described [14]. Cell cultures were passaged weekly and utilized between passages 88 and 99.

Determination of hormonal effects on ZR-75-1 cell proliferation was performed as described previously [14]. Briefly, ZR-75-1 stock cell cultures in late exponential growth phase were harvested with 0.05% trypsin/0.02% EDTA (w/v) and resuspended in phenol red-free RPMI 1640 supplemented as indicated above, except that E$_2$ was omitted and the serum supplement was replaced by 5% (v/v) dextran-coated charcoal-treated fetal bovine serum (SD medium). The cell suspension was seeded into Linbro plastic 24-well culture plates (2 cm$^2$/well) at a final density of $4 \times 10^3$ cells/cm$^2$. After 48 h, treatments were initiated by the addition of the indicated concentrations of hormone and/or antihormone to fresh SD medium. Steroids were added from 1000× to 10,000×-concentrated stock solutions in 99% redistilled ethanol, while control cell cultures received the ethanol vehicle only (<0.12% [v/v]), which had no detectable effect on cell growth or morphology. Bovine insulin was added from 100×-concentrated stock solutions in 0.1 N acetic acid. Incubation under the indicated treatment conditions was carried out for 12 days, with refeeding every other day. Cells were harvested at the end of treatment and cell number was...