

Answer 1:

Bibliographic Information

The effect of second-line antiestrogen therapy on breast tumor growth after first-line treatment with the aromatase inhibitor letrozole: long-term studies using the intratumoral aromatase postmenopausal breast cancer model. Long, Brian J.; Jelovac, Danijela; Thiantanawat, Apinya; Brodie, Angela M. Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD, USA. *Clinical Cancer Research* (2002), 8(7), 2378-2388. Publisher: American Association for Cancer Research, CODEN: CCRE4 ISSN: 1078-0432. Journal written in English. CAN 138:147321 AN 2002:601246 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

Abstract

The aromatase inhibitors letrozole and anastrozole were approved recently as 1st-line treatment options for hormone-dependent advanced breast cancer. Although it is established that a proportion of patients who relapse on 1st-line tamoxifen therapy show additional responses to aromatase inhibitors, it was not determined whether tumors that acquire resistance to aromatase inhibitors in the 1st line remain sensitive to 2nd-line therapy with antiestrogens. The aim of this study was to determine whether aromatase-transfected and hormone-dependent MCF-7Ca human breast cancer cells remain sensitive to antiestrogens after: (a) long-term growth in steroid-depleted medium in vitro; and (b) long-term treatment with the aromatase inhibitor letrozole in vivo. In the 1st approach, a variant of the MCF-7Ca human breast cancer cell line was selected that had acquired the ability to grow in estrogen-depleted medium after 6-8 mo of culture. Steroid-deprived UMB-1Ca cells were analyzed for aromatase activity levels, hormone receptor levels, and sensitivity to estrogens and antiestrogens in vitro and in vivo. In the 2nd approach, established MCF-7Ca breast tumor xenografts were treated with letrozole 10 µg/day for 12 wk followed by 100 µg/day for 25 wk until tumors acquired the ability to proliferate in the presence of the drug. Long-term letrozole-treated tumors were then transplanted into new mice, and the effects of antiestrogens and aromatase inhibitors on tumor growth were determined. Steroid-deprived UMB-1Ca breast cancer cells continued to express aromatase activity at levels comparable with the parental cell line. However, compared with MCF-7Ca cells, UMB-1Ca cells expressed elevated levels of functionally active estrogen receptor. The growth of UMB-1Ca cells in vitro was inhibited by the antiestrogens tamoxifen and flutamide and tumor growth in vivo was inhibited by tamoxifen. In the 2nd approach, the time for MCF-7Ca tumor xenografts to approximately double in volume

after being treated sequentially with the increasing doses of letrozole was 37 wk. Long-term letrozole-treated tumors continued to express functionally active aromatase. When transplanted into new mice, growth of the long-term letrozole-treated tumors was slowed by tamoxifen and inhibited more effectively by flutamide. Tumor growth was refractory to the aromatase inhibitors anastrozole and formestane but, surprisingly, showed sensitivity to letrozole. Steroid-deprived UMB-1Ca human breast cancer cells selected in vitro and long-term letrozole-treated MCF-7Ca breast tumor xenografts remain sensitive to 2nd-line therapy with antiestrogens and, in particular, to flutamide. This finding is associated with increased expression of functionally active estrogen receptor after steroid-deprivation of MCF-7Ca human breast cancer cells in vitro.

Answer 2:

Bibliographic Information

Aromatase inhibitors in breast cancer. Brodie, Angela. Dept Pharmacology and Experimental Therapeutics, University of Maryland, School of Medicine, Baltimore, MD, USA. *Trends in Endocrinology and Metabolism* (2002), 13(2), 61-65. Publisher: Elsevier Science Ltd., CODEN: TENME4 ISSN: 1043-2760. Journal; General Review written in English. CAN 137:118895 AN 2002:122086 CAPLUS (Copyright (C) 2008 ACS on SciFinder (R))

Abstract

A review. Several compounds that selectively inhibit estrogen synthesis via aromatase have been developed. Steroidal substrate analogs, such as formestane and exemestane, inactivate aromatase by binding irreversibly to it. Non-steroidal inhibitors, such as the triazole compounds letrozole and anastrozole, are highly potent, reversible inhibitors with good specificity for aromatase. The intratumoral

aromatase model for postmenopausal breast cancer has been used to investigate the efficacy of letrozole, anastrozole and exemestane in combination and sequentially. Combining letrozole or arimidex with tamoxifen or faslodex was not more effective than the aromatase inhibitors alone, but was more effective than tamoxifen alone. Letrozole was superior to and longer lasting than the other agents, suggesting that aromatase inhibitors control tumor growth effectively by inducing greater tumor response and extending treatment time. In addn., aromatase inhibitors can be effective in patients relapsing from tamoxifen. Because two types of aromatase inhibitors are available, steroidal enzyme inactivators and reversible non-steroidal inhibitors in sequential therapy could be useful if resistance to one type develops. Aromatase (estrogen synthesis) inhibitors have been developed. These are more effective than tamoxifen in mouse xenograft models, and current clin. data suggest the inhibitors are likely to improve breast cancer treatment.

Answer 3:

Bibliographic Information

Preclinical evaluation of aromatase inhibitors antitumor activity. Auvray P; Bichat F; Genne P Oncodesign Biotechnology, Parc technologique de la Toison-d'Or, 28, rue de Broglie, 21000 Dijon, France Bulletin du cancer (2000), 87 Spec No 7-22. Journal code: 0072416. ISSN:0007-4551. (ENGLISH ABSTRACT); Journal; Article; (JOURNAL ARTICLE); General Review; (REVIEW) written in French. PubMed ID 11250604 AN 2001184265 MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

Abstract

Aromatase is an enzymatic complex responsible for the conversion of androgens into estrogens; these hormones are important in development, reproduction, but also in the growth of estrogen-dependent cancer. This enzyme is present in 60-70% of the breast cancer. The aromatase inhibitors are important drugs in the breast cancer treatment of postmenopausal women. In order to study their in vivo activity, animal models have been developed, e.g. rat with tumour induced by 7,12-dimethylbenz[a]anthracene, PMSG-primed immature rat or athymic nude mice with aromatase transfected MCF-7 xenograft. In this review, we were interested in preclinical results obtained with both classes: steroidal and nonsteroidal inhibitors. The former group, as substrate analogs formestane or exemestane, are irreversible, selective and long-lasting inhibitors of aromatase. The nonsteroidal molecules, such as letrozole or anastrozole, are reversible inhibitors with high affinity. Finally, knowledge of the enzyme active site, with molecular modeling and site-directed mutagenesis, could be useful to develop new inhibitor families, more specific and potent in vivo.

Answer 4:

Bibliographic Information

Tamoxifen-resistant fibroblast growth factor-transfected MCF-7 cells are cross-resistant in vivo to the antiestrogen ICI 182,780 and two aromatase inhibitors. McLeskey S W; Zhang L; El-Ashry D; Trock B J; Lopez C A; Kharbanda S; Tobias C A; Lorant L A; Hannum R S; Dickson R B; Kern F G Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC 20007, USA Clinical cancer research : an official journal of the American Association for Cancer Research (1998), 4(3), 697-711. Journal code: 9502500. ISSN:1078-0432. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T); (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.); (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.) written in English. PubMed ID 9533540 AN 1998192211 MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

Abstract

Although the antiestrogen tamoxifen has been the mainstay of therapy for estrogen receptor (ER)-positive breast cancer, successful treatment of responsive tumors is often followed by the acquisition of tamoxifen resistance. Subsequently, only 30-40% of patients have a positive response to second hormonal therapies. This lack of response might be explained by mechanisms for tamoxifen resistance that sensitize ER pathways to small amounts of estrogenic activity present in tamoxifen or that bypass ER pathways completely. To elucidate one possible mechanism of tamoxifen

resistance, we treated ovariectomized tumor-bearing mice injected with fibroblast growth factor (FGF)-transfected MCF-7 breast carcinoma cells with the steroidal antiestrogen ICI 182,780 or one of two aromatase inhibitors, 4-OHA or letrozole. These treatments did not slow estrogen-independent growth or prevent metastasis of tumors produced by FGF-transfected MCF-7 cells in ovariectomized nude mice. FGF-transfected cells had diminished responses to ICI 182,780 in vitro, suggesting that autocrine activity of the transfected FGF may be replacing estrogen as a mitogenic stimulus for tumor growth. ER levels in FGF transfectants were not down-regulated, and basal levels of transcripts for estrogen-induced genes or of ER-mediated transcription of estrogen response element (ERE) luciferase reporter constructs in the FGF expressing cells were not higher than parental cells, implying that altered hormonal responses are not due to down-regulation of ER or to FGF-mediated activation of ER. These studies indicate that estrogen independence may be achieved through FGF signaling pathways independent of ER pathways. If so, therapies directed at the operative mechanism might produce a therapeutic response or allow a response to a second course of antiestrogen treatment.

Answer 5:

Bibliographic Information

The control and biological importance of intratumoural aromatase in breast cancer. Dowsett M; Lee K; Macaulay V M; Detre S; Rowlands M; Grimshaw R Royal Marsden Hospital, London, U.K The Journal of steroid biochemistry and molecular biology (1996), 56(1-6 Spec No), 145-50. Journal code: 9015483. ISSN:0960-0760. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, NON-U.S. GOV'T); General Review; (REVIEW) written in English. PubMed ID 8603035 AN 96184198 MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

Abstract

The existence of aromatase activity in human breast carcinomas has been established for about 20 years but the clinical and biological importance of this remains unclear. A number of studies in clinical material suggest that aromatase activity may be a prerequisite of response to aromatase inhibitors and that aromatase activity may be enhanced in those tumours relapsing during treatment with one such inhibitor, aminoglutethimide. These results would carry more significance, however, if it was demonstrable that the growth of breast carcinomas is affected by the conversion of androgens to oestrogens by intratumoural aromatase. We have tried to address this by establishing model systems with aromatase-transfected MCF7 breast cancer cells. We have demonstrated that these cells can be stimulated mitogenically with androgen and that this proliferation is suppressible with aromatase inhibitors. Similarly the growth of aromatase transfected cells but not wild type cells as xenografts is supported by androstenedione and inhibitable by both the steroidal aromatase inhibitor, 4-hydroxyandrostenedione and non-steroidal inhibitor, CGS 20267. Work with the former of these, which is a suicide inhibitor allowed us to demonstrate that growth can proceed with aromatase activity approximating to the highest level seen in breast carcinomas indicating that at least at this extreme level the intratumoural conversion of androgens to oestrogens may indeed be able to support tumour growth. Further work with this model system should allow us to define the minimal amount of intratumoural activity which can support tumour growth.

Answer 6:

Bibliographic Information

MCF-7 human breast carcinomas in nude mice as a model for evaluating aromatase inhibitors. Yue W; Brodie A Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore 21201 The Journal of steroid biochemistry and molecular biology (1993), 44(4-6), 671-3. Journal code: 9015483. ISSN:0960-0760. Journal; Article; (JOURNAL ARTICLE); (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.) written in English. PubMed ID 8476781 AN 93237152 MEDLINE (Copyright (C) 2008 U.S. National Library of Medicine on SciFinder (R))

Abstract

While hormone-dependent, mammary tumors induced with carcinogens (DMBA or NMU) in intact rats have been used extensively for studying aromatase inhibitors, there is currently no suitable model to investigate their effects in human breast cancers *in vivo*. While hormone responsive tumors can be formed in the athymic mouse using human breast carcinoma MCF-7 cells, due to the low ovarian estrogen production, tumor growth is induced with estradiol supplementation. Thus, this model is unsuitable for studies of aromatase inhibitors. We have induced tumors without the need for estrogen supplementation by co-inoculating MCF-7 cells with Matrigel, a basement membrane preparation, into intact athymic mice. In one experiment, 45 days after inoculation, mice were assigned to the control group or 4-hydroxyandrostenedione (4-OHA) (1 mg/day s.c.) treatment for 52 days. Tumor volumes in the control mice increased 672%, whereas tumor volumes in the treated mice did not change significantly (178.9 +/- 16.2 to 336.6 +/- 120 mm³). In the second experiment, 55 days after inoculation, groups of mice were treated with the antiestrogen, tamoxifen (5 micrograms/day s.c.) or vehicle (controls). Tumor volumes in the control mice increased 325% in 58 days, whereas there was no significant change in tumor volume in the tamoxifen treated group (338.8 +/- 55.3 to 330.6 +/- 84.9 mm³). The results suggest that (1) the tumors resulting from MCF-7 cells co-inoculated with Matrigel are estrogen-dependent and (2) tamoxifen and 4-OHA were effective in suppressing growth of these tumors. The results suggest that this model should be useful for evaluating the effects of aromatase inhibitors and for comparing breast cancer treatments.