Original Articles

Progesterone receptor antagonism inhibits progestogen-related carcinogenesis and suppresses tumor cell proliferation

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ABSTRACT

Purpose: Blockade of the progestogen–progesterone receptor (PR) axis is a novel but untested strategy for breast cancer prevention. We report preclinical data evaluating telapristone acetate (TPA), ulipristal acetate (UPA), and mifepristone.
Methods: Tumors were induced with medroxyprogesterone acetate (MPA) plus 7,12-dimethylbenz[a]anthracene (DMBA) in mice, and MPA or progesterone plus N-methyl-N-nitrosourea (MNU) in rats. Mammary gland histology, tumor incidence, latency, multiplicity, burden and histology were evaluated, along with immunohistochemical labeling of pHH3 (proliferation), CD34 (angiogenesis), and estrogen and progesterone receptors (ER and PR). A concentration gradient of TPA, UPA, and mifepristone was tested for growth inhibition of T47D spheroids.
Results: In mouse mammary glands, no tumors formed, but TPA opposed the pro-hyperplastic effects of MPA (p = 0.002). In rats, TPA decreased tumor incidence (p = 0.037 for MPA + TPA vs. MPA, and p = 0.032 for progesterone + TPA vs. progesterone) and tumor burden (p = 0.042 for progesterone + TPA vs. progesterone), with significant decreases in pHH3 and CD34 positive cells. TPA and UPA were superior to mifepristone in growth inhibition of T47D spheroids.
Conclusion: TPA has consistent anti-tumorigenic effects in several models, which are accompanied by decreases in cell proliferation, angiogenesis, and hormone receptor expression.

Introduction

Breast cancer causation has been linked to repeated exposure of the breast to estrogen and progesterone, producing waves of epithelial proliferation in the luteal phase of the menstrual cycle [1–4]. More sustained exposure with estrogen plus MPA increases breast cancer risk to a greater extent than estrogen alone in postmenopausal women [5,6], as do progestin-based contraceptives in premenopausal women [7–9]. Moreover, the tumors seen with the use of depot MPA and combined hormone therapy appear to be more aggressive [8,10]. Additionally, MPA and progesterone augment tumor formation in DMBA-treated rats [11]. Concomitantly, the role of PR in breast carcinogenesis is increasingly recognized [12–14]. These data point to PR blockade as an excellent (but clinically untested) strategy for breast cancer prevention.

Second generation anti-progestins are under development for the treatment of benign gynecological conditions [13,15–17], thereby providing robust toxicity data for the design of breast cancer prevention trials [13,16,18,19]. These agents differ from mifepristone (RU486) and onapristone, which were tested in postmenopausal metastatic breast cancer patients, but caused liver toxicity and anti-glucocorticoid effects [20–23]. The second generation anti-progestins are potent, and preserve PR selectivity while reducing the 'off-target' effects on glucocorticoid receptor (GR), and androgen receptor (AR), which are seen with mifepristone [24–27]. Among these, TPA (CDB-4124) and UPA (CDB-2914) have attracted attention as potential breast cancer treatment and prevention agents, based on preclinical efficacy data [25,26,28–30] and a good safety profile. Additional rationale for a focus on prevention derives from data pointing to involvement of the progesterone-pathway in early steps in cancer development [12–14]; in contrast, the data on the treatment of an advanced disease are confusing, since high doses of megestrol acetate [31–33] and MPA [34,35] have therapeutic efficacy–this finding is similar to high dose estrogen (HDE) [36,37]. Previous studies show that TPA significantly delays the growth of established DMBA-induced mammary tumors and delays tumor onset in MNU-treated rats [28,29]. We now report experiments addressing the anti-cancer efficacy of TPA in rodents exposed to carcinogens plus progestogens (MPA or progesterone), and growth suppression of T47D spheroids grown in physiological estradiol and

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progesterone concentrations. Our goal was to develop biomarkers of response for use in our ongoing trials (clinicaltrials.gov NCT01800422 and NCT02314156), and test the hormonal conditions under which anti-progestins are effective in repressing tumor cell growth. We confirm prior studies suggesting that both proliferation and angiogenesis are decreased by anti-progestins [29], and find that TPA, UPA, and mifepristone are effective in suppressing the growth of T47D spheroids in both premenopausal and postmenopausal hormone conditions.

Materials and methods

Materials

17β estradiol, progesterone, mifepristone, DMBA, MNU, and sesame oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). TPA and UPA were a gift from Repros Therapeutics, Inc. (The Woodlands, TX) and HRA-Pharma (Paris, France), respectively. Subcutaneous pellets (90 day release) of progesterone, MPA, and TPA were manufactured by Innovative Research of America, Inc. (Sarasota, Florida).

Treatment of DMBA-induced mammary carcinogenesis in mice

All procedures followed the Animal Care and Use Committee Guidelines of Northwestern University (see Fig. 1, legends for detail).

Treatment of MNU-induced carcinogenesis in rats

All procedures followed the Animal Care and Use Committee Guidelines of Northwestern University. A single intraperitoneal injection of MNU (50 mg/kg body weight) was given to all rats at the age of 4–5 weeks [38], followed by randomization to five treatment groups: control, MPA, progesterone, MPA + TPA, and progesterone + TPA (see Fig. 2, legends for detail).

Histopathology and immunohistochemistry (IHC)

FFPE samples of mouse and rat mammary glands and tumors were sectioned at 5 microns, stained with hematoxylin and eosin (H&E) for histological evaluation, and immunostained for evaluation of epithelial cell proliferation by phosphohistone domain H3 (pHH3) staining, angiogenesis by CD34 expression of tumor endothelial cells, and modulation of estrogen receptor α (ER) and progesterone receptor (PR). All the staining was performed with standard procedures by the Mouse Histology & Phenotyping Laboratory at the Northwestern University.

Mouse and rat mammary glands and tumor morphologies were determined blindly by a single pathologist (KC) as suggested by Russo et al. [39], noting histological changes in mammary glands and the presence of invasive and in situ tumors (Figs. 1–3).

Hormones and TPA measurements

Rat blood samples were collected by cardiac puncture from animals at euthanasia. Serums for estradiol and progesterone and plasma for TPA were assayed by liquid chromatography and tandem mass spectroscopy (LC–MS/MS).
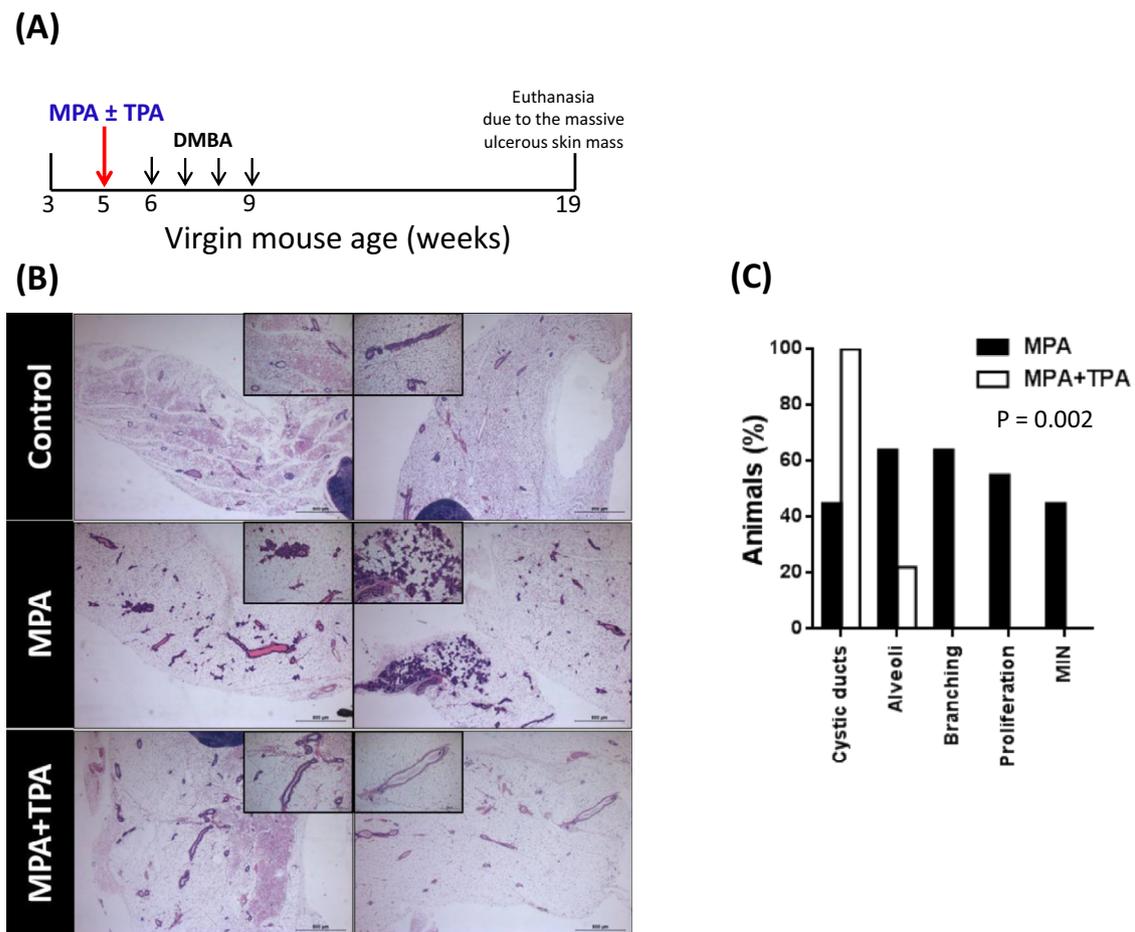

Fig. 1. Effects of MPA and TPA on DMBA-induced carcinogenesis in mice. (A) Animal study protocol. Three-week old female FVB/NHsd mice (Harlan Laboratories, Madison, WI) were housed in a temperature-controlled room with 12-h light/dark schedule, and provided food (Teklad 8640; Teklad, Madison, WI) and water ad libitum before undergoing experimental procedures. Ovary-intact mice were randomized to control (n = 2), MPA (n = 10) and MPA + TPA (n = 11). MPA (25 mg/pellet) and TPA pellets (25 mg/pellet) were implanted subcutaneously in the lateral neck at 5 weeks of age. One week later, DMBA treatment was initiated: 1 mg in 0.2 mL of sesame oil given by gavage and continued weekly for 4 weeks. The mice were monitored twice weekly for tumor formation; by 19 weeks of age, they had developed massive ulcerated skin masses and were euthanized. Tumors and mammary glands were formalin-fixed and paraffin-embedded (FFPE). (B) Representative cross-sectional H&E images (2.5× magnification) of mammary glands of DMBA control (top panel), MPA (middle panel), and MPA + TPA treated mice (bottom panel). The representative images were taken from two different animals per treatment group (left and right columns). Inset images were taken at 10× magnification. (C) Summary of histology evaluation of mammary glands showed that MPA treated mice have more alveoli, ductal/alveolar proliferation, and mammary intraepithelial neoplasia (MIN). Significance of overall histological difference between MPA and MPA + TPA groups was calculated with 5 × 2 Chi-square test (p = 0.002).