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Breastdefend Enhances Effect of Tamoxifen in Estrogen Receptor-Positive Human Breast Cancer in Vitro and in Vivo

Isaac Eliaz [1]*, Dan Sliva [2,3]

[1] *Amitabha Medical Clinic and Healing Center, 398 Tesconi Ct., Santa Rosa, CA 95401*

[2] *Daniel Sliva, PhD, Cancer Research Laboratory, Methodist Research Institute, Indiana University Health, Indianapolis, IN 46202, USA*

[3] *DSTest-Laboratories, Purdue Research Park, 5225 Exploration Drive, Indianapolis, IN 46241, www.dstest-lab.com*

** Corresponding author: Isaac.eliaz@gmail.com*

Abstract

Background: Breast cancer is the leading cause of cancer death in females, and approximately 75% are estrogen receptor (ER)-positive breast tumors. Tamoxifen (TAM) has been a frontline treatment for both early and advanced ER-positive breast cancer in pre- and postmenopausal women. TAM is a selective ER modulator and its active metabolite, 4-hydroxytamoxifen (4-OHT), acts as an estrogen antagonist in breast cells that binds ER and blocks its activity to halt cell proliferation and induce apoptosis. Unfortunately, TAM resistance occurs in approximately 30% of patients. Additionally the development of side effects with long-term administration, including menopausal symptoms, fatigue, painful joints and mood changes, significantly affects quality of life and often leads to discontinuation of treatment. TAM in combination with other therapies is being actively investigated as a way to increase efficacy and decrease side effects. Numerous studies have demonstrated that natural compounds present in vegetables, fruits and mushrooms can affect multiple molecular targets and signaling pathways, leading to their possible use in combination therapy. BreastDefend® (BD) is a dietary supplement formula which contains a combination of medicinal mushrooms (Ganoderma lucidum, Coriolus versicolor, Phellinus linteus), herbal extracts (Curcuma longa, Scutellaria barbata, Astragalus membranaceus), and purified biologically active components (3, 3′ -diindolylmethane, quercetin). These natural agents have demonstrated anticancer activities against breast cancer in multiple studies. Prior studies on BD alone or combined with modified citrus pectin (MCP) showed significant inhibition of growth and invasive behavior of highly metastatic triple-negative human breast cancer cells in vitro and in vivo.

Study Purpose: We investigated the sensitivity of ER-positive MCF-7 cells and its tumor xenografts to BD, 4-OHT/TAM and their combination treatment.

Methods: In vitro: Cell proliferation and apoptosis determined in ER-positive human breast cancer cells (MCF-7) by MTT assay, quantitation of cytoplasmic histone-associated DNA fragments and expression of cleaved PARP, respectively. Molecular mechanism identified using RNA microarray analysis and western blotting.

In vivo: MCF-7 cells implanted (SQ) into inguinal mammary fat pads. E2 pellets (0.36 mg/pellet, 60-day release) also implanted. Mice with palpable tumors (~60 mm3) were randomly assigned to four groups (n = 13): control, TAM, BD, and TAM+BD (16–24 tumors/ group). TAM pellets (5 mg/pellet, 60-day release) were implanted (SQ). BD was suspended in water, administered by gavage 5 x/week (100 mg/kg) x 4 weeks. Tumor tissues analyzed by immunohistochemistry.

Results: Data clearly demonstrate that a combination of TAM with BD led to profound inhibition of cell proliferation and induction of apoptosis in MCF-7 cells, consistent with regulation of apoptotic and TAM resistant genes at the transcription and translation levels. In the xenograft model, TAM and BD co-treatment significantly enhanced apoptosis, suppressed tumor growth and reduced tumor weight.

Conclusions: We show, for the first time, that BD and TAM work synergistically against ER positive breast cancer by suppressing estradiol-induced proliferation of MCF-7

Cells in vitro and tumor growth in vivo, by inducing apoptosis and regulating expression of TAM resistant proteins (p21/CDKN1A and Bcl-2). The findings reveal a novel potential strategy against ER-positive human breast cancer using combination treatment of tamoxifen with BreastDefend.

Keywords:

Breast Cancer,

Vitro,

Vivo,

Tamoxifen.