Report

Alteration of the effects of cancer therapy agents on breast cancer cells by the herbal medicine black cohosh

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Summary
Recent studies have revealed that many, perhaps most, patients receiving cancer therapy are simultaneously self-medicating with one or several complementary and alternative medicines, often without discussing the use of these agents with their physicians. The effects of these agents on the efficacy and toxicity of standard anticancer therapy have not been studied. The experiments described in this report used a well characterized mouse breast cancer cell line to ask whether commercially available extracts of black cohosh, an herb widely used by breast cancer patients, altered the response of cancer cells to radiation and to four drugs commonly used in cancer therapy. The black cohosh extracts increased the cytotoxicity of doxorubicin and docetaxel and decreased the cytotoxicity of cisplatin, but did not alter the effects of radiation or 4-hydroperoxycyclophosphamide (4-HC), an analog of cyclophosphamide which is active in cell culture. These data sound a warning that the herbal medicines being used by patients undergoing cancer therapy can have effects on cancer cells that alter their response to the agents commonly used to treat breast cancer.

Introduction
The use of complementary and alternative medicines (CAM) has increased dramatically in recent years [1–5]. Over 30 insurers now cover at least 1 alternative therapy. Out-of-pocket spending for CAM was recently estimated to be $27 billion per year, similar to the out-of-pocket costs for all physician services [1–3].

Many cancer patients use CAM during treatment with conventional cancer therapy [6–18]. It is difficult to establish the exact prevalence of CAM use by cancer patients because of the wide variation in the definitions of CAM used in different surveys, some of which include prayer, support groups, massage, exercise, and other lifestyle factors, as well as topical or ingested herbs, extracts, vitamins, and nutritional supplements. A survey of patients in clinical trials at NIH reported that 63% used at least one form of CAM, with an average of two CAM per patient [11]. A study of women being treated for early stage breast cancer showed that 10.6% had been using one or more CAM at the time of diagnosis, while an additional 28.1% began using CAM after surgery [9]. Many patients do not discuss their use of CAM with their physicians [4, 15, our own observations], often because the physicians do not inquire specifically about CAM and nutritional supplements or indicate that they only need information on prescription medications. CAM have been associated with adverse effects, including drug interactions [2, 15–20]. The possibility of interactions between CAM and conventional medical treatments is therefore of concern.

Black cohosh is one of the agents most commonly mentioned by breast cancer patients as being used during radiotherapy and chemotherapy. Black cohosh (Cimicifuga racemosa) [21–23] is a shrub-like plant native to the eastern forests of North America. It was used for centuries by Native American herbalists for menopausal symptoms, pre-menstrual discomfort and dysmenorrheas, to induce abortion, and for a variety of other indications. The herb was listed in the Pharmacopoeia during the 19th century and was a major constituent of the once popular patent medicine ‘Lydia Pinkham’s Vegetable Compound’. A variety of black cohosh preparations are available from drug stores, herbalists and traditional healers and recommended by these sources as being safe, effective natural remedies for menopausal symptoms. Black cohosh is being used by women who have been advised by their physicians to avoid HRT, who are at high risk for breast cancer or who have discontinued HRT after a diagnosis of breast cancer.

The rigorous scientific literature on black cohosh is surprisingly sparse. Most studies have focused on the herb’s effects on menopausal symptoms [24, 25]. The active component(s) have not been definitively
identified; triterpene glycosides (including 27 deoxyacetic, acetin, and cinobufagin), have been hypothesized to be the critical components, but resins and caffeic, isoflavonic and fukinolic acid also have been suggested to have biological activities [26, 27]. There is considerable debate about whether the herb has estrogenic or antiestrogenic activities [28–32] and there are studies in the literature supporting each effect. Our literature searches revealed only a few studies [31–34] testing the effects of black cohosh on breast cancer cells; these gave conflicting findings, with some reporting increases and others no changes or decreases in the growth of breast cancer cells in culture. We found no studies of the interactions of black cohosh with radiation or the drugs used to treat breast cancer patients, except for a study showing that black cohosh had antiestrogen effects which added to the effects of tamoxifen in reducing the proliferation of ER \(^+\)/PR \(^+\) MCF7 breast cancer cells in vitro [34]. To investigate the potential clinical significance of the use of black cohosh during cancer treatment, we asked whether black cohosh altered the response of breast cancer cells in cell culture to radiation and to some widely used anticancer drugs.

**Methods**

**Biological system**

All studies were performed using EMT6 mouse mammary tumor cells. Growth of this well-characterized, undifferentiated breast cancer cell line is not dependent on estrogen or progesterone [35–37]. This model was used to test for effects of black cohosh above and beyond the estrogenic/antiestrogenic effects reported by others [27–32]. EMT6 cells in culture are grown in Waymouth’s medium supplemented with 15% serum and antibiotics, as described in detail previously [36].

**Cell growth and viability**

Exponentially growing cultures were trypsinized, and the cells were suspended and plated at \(5 \times 10^5\) cells per dish in Petri dishes containing 5 ml of medium. Black cohosh extracts were added to the culture medium 4 h after subculture. Each day, two cultures from each group were selected, the cells were suspended and counted, and the number of cells per dish was calculated. Cells from one dish per group were plated to assess the viability of the cells using a rigorous clonogenic assay, described in detail elsewhere [37], in which known numbers of cells were plated and allowed to grow for 2 weeks to form macroscopic colonies, which were fixed and stained and counted. All growth and viability studies were performed at least twice.

**Cell survival curves**

To measure the effect of black cohosh on the survival of cells treated with radiation or drugs, cultures were established by plating \(2 \times 10^5\) cells into dishes and incubating the cultures for 3 days before treatment. Cells were then suspended, and assayed for colony formation. Surviving fractions were calculated relative to untreated control cultures from the same experiments; approximately 60% of the cells in untreated cultures formed colonies. Cultures were treated with black cohosh for 4 h before radiation or drug treatment, throughout the drug treatment, and after treatment for a total of 24 h. This regimen was used in these screening studies to ensure that the studies would detect not only effects of black cohosh on such processes as uptake or activation of the drug or the induction of cytotoxic damage by the drug (which would be seen with pre-treatments and simultaneous treatments), but also effects on drug efflux or on repair of damage (which would occur after drug treatment). Treatments expected to reduce cell survival to approximately 1% of the control were used, except for docetaxel, where the resistance of the EMT6 cells prevented attaining low survivals.

**Radiation and drugs**

Cells were irradiated using 250 kVp X-rays (15 mA, 2 mm Al equivalent filtration) produced by a Siemen’s Stabilipan at a dose rate of 1.1 Gy/min. Doxorubicin was from American Pharmaceutical Partners. Docetaxel was from Adventis Pharmaceuticals. Cisplatin was from Sigma. 4-HC was obtained from Dr. Susan Ludeman at Duke University.

**Black cohosh extracts**

Our initial studies examined the effects of a ‘standardized’ liquid extract of black cohosh from GAIA Herbs purchased from a local store. This was chosen for our pilot studies because it is commercially available in Connecticut and because its liquid formulation in ~50% ethanol and ~50% water facilitated the experiments. This extract is described on the label as standardized to contain 3.0% triterpene glycosides ‘providing 1.2 mg + of bioactivity per dose’. Subsequent experiments used two other liquid black cohosh extracts, purchased from local stores: a GNC extract (which was labeled ‘Herbal Plus\(^{\text{TM}}\) standardized black cohosh’ and described as containing 2.5% triterpene glycosides, providing 1 mg/dose), and a Nature’s Answer extract (described on the label as ‘guaranteed to contain 2 mg triterpene glyco deoxyactein and 1 mg isoflavonoids as formononetin’). Because the active ingredient(s) of these extracts is unknown and because the composition and/or potency could differ for different extracts, the amount of each extract used in the experiments was determined by taking the total daily dose of extract recommended on