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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 deoxyactein, acetein, and cimifugoside), have been hypothesized to be the critical components, but resins and caffeic, isoferulic 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 5×10^4 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 2×10^5 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 pretreatments 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 A1 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® 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 isoflavenoids 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

the label, assuming that this dose was uniformly distributed in the body of a 'standard' woman, and adding extract to the culture medium to produce this concentration or 10 or 100 times this concentration.

Results

Effect of black cohosh extracts on the growth of breast cancer cells in vitro

None of the black cohosh extracts tested altered the growth or viability of EMT6 cells in cell culture. There were no statistically significant differences between the growth of control cultures, cultures treated with vehicle, and cultures treated with black cohosh extracts at 1, 10 or 100 times the calculated human dose (data not shown). In addition, the colony forming ability of the black cohosh treated cells was not significantly different from that of the control cells at any time, with any extract (data not shown).

Radiation and anticancer drugs

Agents that have no direct effects on the growth or viability of tumor cells can be important in cancer therapy if they alter the response of tumors or dose-limiting normal tissues to the therapeutic agents. We therefore asked whether a high dose of black cohosh extract altered the response of the breast cancer cells to radiation or to four anticancer drugs.

Figure 1 shows data from experiments examining the effect of a high concentration of black cohosh (100 times the recommended dose) on the survival of EMT6 cells treated with doxorubicin. The survival curves for cells treated with doxorubicin alone or doxorubicin plus vehicle were indistinguishable. However, cells treated with black cohosh were more sensitive to the cytotoxic effects of doxorubicin. The separation between the curves increased with increasing dose of doxorubicin, resulting in a 40-fold difference in the surviving fractions of the tumor cells at the highest dose tested.

To assess whether this effect was unique to one commercial extract, we examined the effects of two additional extracts on the survival curves for breast cancer cells treated with doxorubicin (Figure 2). The shapes of the survival curves for cells treated with these extracts were similar to those obtained with the initial extract. However, the magnitude of the sensitizing effect varied for the different extracts; this may reflect differences in the concentration(s) of the active specie(s) in the different extracts.

Further experiments (Figure 3) revealed that the effects of the black cohosh extracts were not limited to the very high doses of black cohosh used in the initial screening experiments. Studies examining the effects of graded doses of black cohosh combined with a constant dose of doxorubicin (1.6 μg/ml for 2 h) revealed a steady decrease in cell survival with increasing black cohosh dose. Although the decrease seen at the recommended dose was not statistically significant, a statistically significant decrease in survival was seen at doses as low as 2.5 times the recommended dose. This is well within the range of black cohosh doses used by some women. Moreover, our preliminary studies have shown that mice can be treated with black cohosh extracts in their

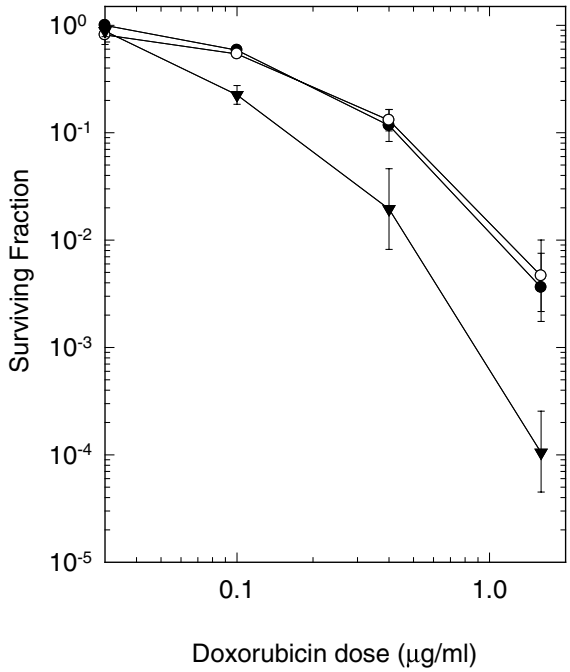

Figure 1. Effect of black cohosh extract on the toxicity of a 2 h doxorubicin treatment to breast cancer cells. (●) Doxorubicin only, (○) vehicle plus doxorubicin, (▼) black cohosh plus doxorubicin. Points are means ± SEMs for three independent experiments.

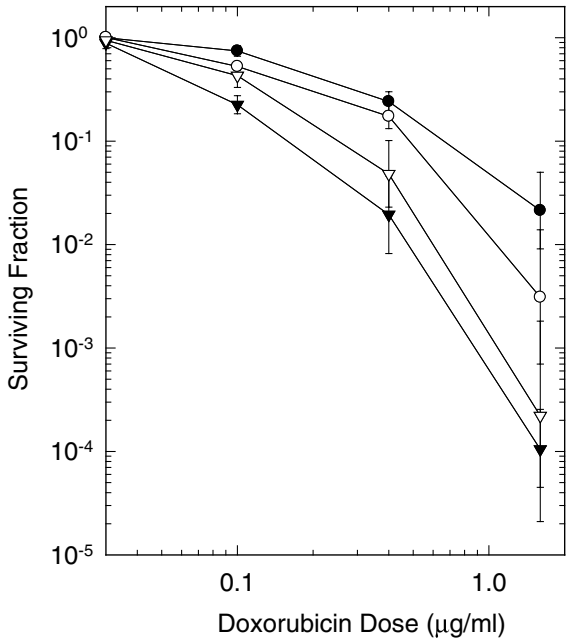

Figure 2. Effects of different commercial black cohosh extracts on the toxicity of a 2 h treatment with doxorubicin. (●) Doxorubicin only, (▼) GAIA extract, (▽) GNC extract, (○) Nature's Answer extract. Points are means ± SEMs for 3–6 independent experiments.

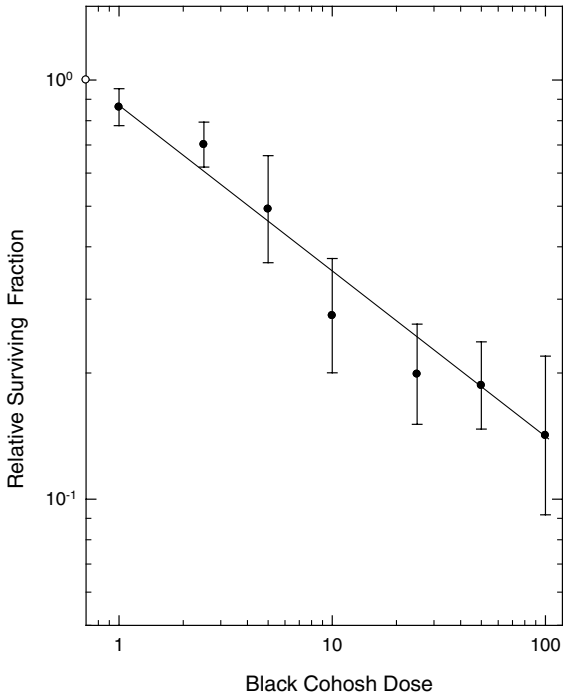

Figure 3. Effect of different doses of black cohosh on the survival of cells treated with 1.6 μg/ml doxorubicin for 2 h. Points are relative cell survivals, normalized to the survivals obtained with vehicle plus doxorubicin within the same experiments, and are means ± SEMs from six independent experiments.

drinking water at doses up to 25 times the human dose for several weeks with no evidence of toxicity (Rockwell and Higgins, unpublished observations). This suggests that sensitization of breast cancer cells to the toxic effects of doxorubicin occurs at doses of black cohosh that are attainable *in vivo*.

Figure 4 shows data from experiments examining the effect of a high concentration of black cohosh on the survival of EMT6 cells treated with radiation, cisplatin, docetaxel and 4-HC. Black cohosh did not alter the survival curve for cultures treated with radiation (panel A). Cells treated with the black cohosh were slightly more resistant to cisplatin than were cells treated with cisplatin alone or cisplatin plus vehicle (panel B); this protection was small, but statistically significant. A 2 h treatment with docetaxel had limited effects on the viability of EMT6 cells even at maximal attainable concentrations (panel C). Cells treated with the black cohosh were more sensitive to the cytotoxic effects of docetaxel than were cells treated with docetaxel alone or docetaxel plus vehicle. This difference was statistically significant and did not increase with increasing docetaxel dose. Black cohosh did not alter the response of cells to 4-hydroperoxycyclophosphamide (4HC), an analog of cyclophosphamide that does not require activation by liver enzymes and therefore is active in cell culture (panel D).

These data show that effects of this herbal extract varied with the therapeutic agent, having no effect on radiation and 4-HC, a slight protective effect for cisplatin, and a significant sensitizing effect for doxorubicin and docetaxel.

Discussion

These experiments are our initial studies in a project examining the effect of a widely used herbal medicine on the growth and response to cancer therapy agents of breast cancer cells *in vitro* and *in vivo*. Our literature search revealed only a few papers [28, 31–34] examining the effects of black cohosh on breast cancer cells; these reported conflicting findings, with some reporting increases and others decreases in cell growth. This is not unexpected, considering the debate about whether the herb has estrogenic or antiestrogenic activities and the studies suggesting each type of effect in different systems [22–32]. Our studies were performed using EMT6 cells, a well-characterized, undifferentiated mouse breast cancer cell line with a growth rate which is not dependent on estrogen or progesterone [35, 36]. This model system was chosen to test for effects of black cohosh above and beyond its estrogenic/antiestrogenic effects. None of the extracts tested altered the growth or viability (colony forming ability) of EMT6 cells.

The studies were performed using commercial black cohosh extracts, chosen because they were stated to be 'standardized'. However, neither the standardization described on the labels nor the effects of the extracts on the response of cells to doxorubicin were identical. The differences in the effects of the extracts may reflect differences in the concentrations of the critical active component(s), which have not been identified. Further studies will be needed to determine which component(s) produce the effects seen in our studies.

The reasons for the different effects of the black cohosh extract on the cytotoxicities of these different anticancer agents is not known. The lack of effect on the cytotoxicity of radiation could indicate that the extract is affecting drug uptake or efflux; if so, there is a differential effect on the transport of the different drugs studied in these experiments. The differences could also reflect the differing mechanisms of action of the agents. Radiation kills cells through relatively non-specific DNA damage. Doxorubicin binds to nucleic acids, presumably through specific intercalation of the planar anthracycline moiety and may produce DNA strand breaks; it may also cause cytotoxicity through effects related to its binding to cell membranes. Docetaxel binds to and stabilizes microtubules and disrupts mitosis and other activities requiring the coordinated disassembly and re-assembly of microtubules. Cisplatin and 4-HC kill cells through DNA crosslinks and adducts. The five agents used in our screening studies have very different properties and mechanisms of action. A variety of mechanisms can be envisioned through which the components of black cohosh could interact with the agents themselves, the metabolic and chemical reactions that lead to the production of damage, the cellular targets, the damaged moieties, or the repair machinery so as to have differential effects on different agents. The mechanism(s) underlying the interactions reported here is a topic that requires further study.

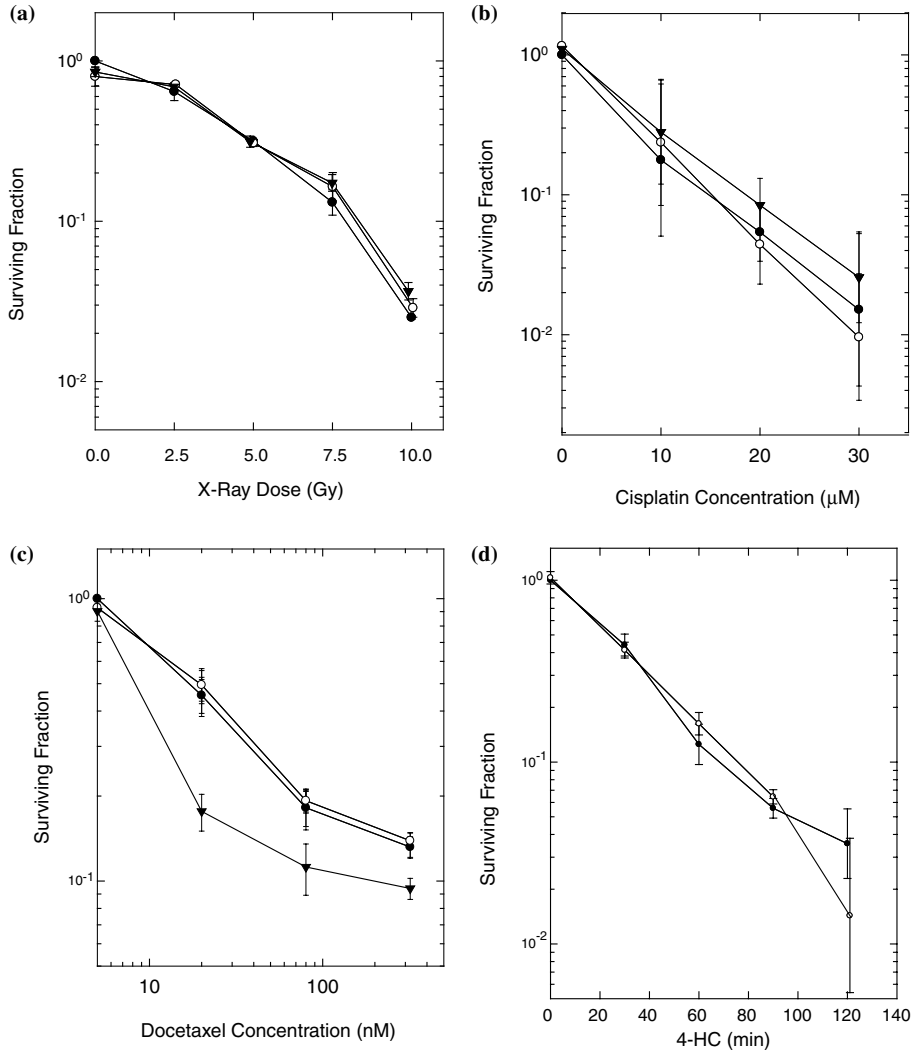

Figure 4. Effect of black cohosh on the response of cells to other agents widely used in cancer therapy. Panel A. Radiation: (●) Radiation only, (○) vehicle plus radiation, (▼) black cohosh plus radiation. Points are means ± SEMs for three independent experiments. Panel B. Cisplatin, 2 h. (●) drug only, (○) vehicle plus drug, (▼) black cohosh plus drug. Points are means ± SEMs for three independent experiments. Panel C. Docetaxel 2 h, ●: drug only. ○: vehicle plus drug. (▼) black cohosh plus drug. Points are means ± SEMs for three independent experiments. Panel D: 4-hydroperoxycyclophosphamide, 20 μM for different periods of time: (●) vehicle plus 4-HC, (○) black cohosh plus 4-HC. Points are means ± SEMs for three independent experiments.

In addition, there are other mechanisms by which black cohosh extracts could alter the effects of anticancer drugs and radiation *in vivo* but which would not have been seen in the cell culture studies reported here. For example, black cohosh extracts could alter the activity of liver enzymes, such as cytochrome p450, and thereby alter the metabolism and effects of anticancer drugs. Therefore, although black cohosh in these cell culture studies did not alter the cytotoxicity of 4-HC (which does not require liver activation), *in vivo* studies will be required to assess whether the extract alters the response of EMT6 breast tumors in mice to cyclophosphamide, a widely used anticancer drug which requires activation by the liver to produce its effects. Similarly, effects of black cohosh extracts on tumor blood flow or tumor oxygenation could result in changes in the response of tumors *in vivo* to radiation, but would not be seen in cell culture. The possibility of such interactions will be examined in our studies with EMT6 tumors in mice.

Further studies are needed to ascertain whether the effects seen *in vitro* also occur *in vivo* and to assess their potential therapeutic implications. These studies, which are now underway under the support of a grant from the NCI, will examine the effect of black cohosh extracts on the therapeutic responses of mouse breast tumor models and of the normal tissues that limit the intensity of cancer therapy. While an increase in the response of tumors to doxorubicin such as that seen here could potentially have therapeutic benefit, an analogous increase in the effect of the drug on marrow or myocardium could produce life-threatening toxicities. Our studies caution that black cohosh should not be considered to be a harmless herb that is inconsequential to the health of cancer patients or to the outcome of con-

ventional cancer therapy. This merits emphasis, because this herb is being widely used by and recommended to breast cancer patients who are experiencing menopausal symptoms due to removal from HRT or to the effects of their therapy. Until the effects of black cohosh are better defined, the use of this and similar herbal preparations by breast cancer patients should be discouraged.

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