Nutrition and Cancer

White Button Mushroom Phytochemicals Inhibit Aromatase Activity and Breast Cancer Cell Proliferation[1,2]

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ABSTRACT Estrogen is a major factor in the development of breast cancer. In situ estrogen production by aromatase/estrogen synthetase in breast cancer plays a dominant role in tumor proliferation. Because natural compounds such as flavones and isoflavones have been shown to be inhibitors of aromatase, it is thought that vegetables that contain these phytochemicals can inhibit aromatase activity and suppress breast cancer cell proliferation. Heat-stable extracts were prepared from vegetables and screened for their ability to inhibit aromatase activity in a human placental microsome assay. The white button mushroom (species *Agaricus bisporus*) suppressed aromatase activity dose dependently. Enzyme kinetics demonstrated mixed inhibition, suggesting the presence of multiple inhibitors or more than one inhibitory mechanism. "In cell" aromatase activity and cell proliferation were measured using MCF-7aro, an aromatase-transfected breast cancer cell line. Phytochemicals in the mushroom aqueous extract inhibited aromatase activity and proliferation of MCF-7aro cells. These results suggest that diets high in mushrooms may modulate the aromatase activity and function in chemoprevention in postmenopausal women by reducing the in situ production of estrogen. J. Nutr. 131: 3288–3293, 2001.

KEY WORDS: • *breast cancer* • *aromatase* • *white button mushrooms* • *diet and cancer prevention*

Breast cancer is the most common cancer affecting women. Despite earlier detection and improved adjuvant therapies, the breast cancer incidence was 109.7 cases per 100,000 women and the death rate was 25.6 per 100,000 women between 1990 and 1997 (1). Emerging evidence suggests that in situ estrogen production in the breast of postmenopausal women may play an important biological role in carcinogenesis (2–7). Aromatase, a cytochrome P_{450} enzyme complex, converts androgens to estrogens. Aromatase expression occurs in breast tumors (2,6) and may play a more dominant role in tumor proliferation than circulating estradiol (8–10).

A major effort to reduce breast cancer mortality and morbidity is focused on developing better breast cancer prevention strategies. Hormonal blockade with tamoxifen has reduced the incidence of invasive and noninvasive breast cancer in high risk women (11). An alternative drug-based strategy for hormonal modulation is the inhibition of aromatase (12). Aromatase inhibitors reduce serum estradiol levels in healthy female volunteers and in animal studies (13–16).

Although pharmaceutical agents have therapeutic and preventative roles in breast cancer, the use of foods and dietary compounds to prevent breast cancer is currently being explored. A variety of complementary substances including plant extracts, fruits and vegetables, vitamins, minerals and minor dietary constituents are undergoing vigorous evaluation as promising sources of anticancer agents (17). Plants contain a variety of compounds that block the formation of carcinogens, alter membrane structure, suppress DNA synthesis, enhance cell differentiation and compete with estrogen for estrogen receptors (18). Cohort and case-controlled epidemiologic studies have investigated the effect of certain vegetables and fruits on the incidence of cancer (18–20). The contribution of fruits and vegetables in relative risk reduction of breast cancer is conflicting and it is difficult to draw absolute conclusions from the current data. A recent meta-analysis did suggest an inverse relationship between intake of fruits and vegetables with the relative risk of developing breast cancer (21). This association was greater for vegetables than for fruits. There is compelling epidemiologic data in Asian women who have a four- to sixfold lower risk of breast cancer than Western women (22). One of the reported differences between these two populations is the consumption of soy protein (23). A possible mechanism-based explanation for the lower incidence of breast cancer is that soy is high in phytoestrogens (24), phytochemicals that suppress aromatase (25–31).

Our laboratory has been interested in the interaction of flavone and isoflavone phytoestrogens with human aromatase and the inhibitory capacity of various compounds (32,33). In the quest for novel compounds, we reported promising data on the effect of grape juice on aromatase inhibition and in vivo suppression of breast cancer cell growth in a nude mouse tumor model (34). These exciting findings have encouraged us to screen a variety of vegetables using aromatase inhibition as the mechanism to identify vegetables that could be a potential

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dietary source for novel aromatase inhibitors. The current study identified the edible mushroom, *Agaricus bisporus*, as a potent inhibitor of aromatase.

MATERIALS AND METHODS

Materials. Androstenedione, testosterone and 4-hydroxy androstenedione (4-OHA)[5] were obtained from Sigma (St. Louis, MO). [1β-^{3}H]androstenedione ([1β-^{3}H]A) was purchased from NEN Dupont (Boston, MA). Heat-stable extracts from green onion, celery, carrot, bell pepper, broccoli, spinach and mushroom were prepared by boiling in water (70 g wet matter/200 mL H_2O) and centrifuging at 35,000 $\times$ *g* for 30 min. The supernatant was Millipore (0.45 μm; Bedford, MA) filtered, concentrated in a Speed-VAC centrifuge and reconstituted in H_2O at 7.5X concentration ($7.5X_{H2O}$). Heat was used to break up the compartmentalization that exists in biological materials (35).

For the kinetic analysis, cell culture studies, step gradient separations and HPLC analysis, stock preparation of the mushroom extract was prepared from fresh, washed white button mushrooms (*Agaricus bisporus*). The lyophilized heat-stable white button mushroom extract was reconstituted as a 10X stock concentrate in water ($10X_{H2O}$) or culture medium ($10X_{CM}$) as indicated below for individual studies.

Aromatase inhibition studies were performed using human placental microsome preparations, which contain a high level of aromatase. Microsomes were prepared from human placenta as previously described (36,37). The MCF-7 cell line, a human estrogen receptor positive (ER^+) adenocarcinoma, was obtained from ATCC (Manassas, VA). MCF-7 cells transfected with human aromatase (MCF-7aro) were generated by stable transfection of MCF-7 cells with the human placenta aromatase cDNA under the control of the β-actin promoter (38).

Placental microsome assay. Aromatase converts androgens to estrogens through three hydroxylations (39). The third hydroxylation is thought to involve position C1 of androgens, which leads to aromatization of the A ring of the steroids. The aromatase assay was performed in the following manner. The substrate, androst-4-ene-3, 17-dione [1β-^{3}H(N)] (specific activity 37 MBq/mol) was dissolved in serum-free cell culture medium, millipore filtered and added to the assay mixture. The assay mixture (500 μL) consisted of 20 μg placental microsomes, 100 nmol/L [^{3}H]-androstenedione, 10 μmol/L progesterone, 1 g/L bovine serum albumin and 67 mmol/L potassium phosphate buffer, pH 7.4 (34). After a 10-min preincubation at 22°C, 12 mmol/L NADPH (50 μL) was introduced and the reaction was continued at 37°C for an additional 20 min in the pilot vegetable and mushroom screening experiments. The incubation time was reduced to 10 min for all of the experiments with the white button mushroom. The enzymatic activity was terminated with 5% trichloroacetic acid. One molecule of [^{3}H]-H_2O is formed when one molecule of androstenedione is converted to estrone. The supernatant containing the aromatase product, [^{3}H]-H_2O, was extracted with chloroform and with dextran-treated charcoal. The suspension was centrifuged (700 $\times$ *g* for 10 min) and an aliquot of the supernatant was counted for radioactivity. Aromatase activity was calculated as pmol [^{3}H]-H_2O formed/(mg protein · h). Analyses were performed in triplicate and the data are expressed as the mean ± SD. The [^{3}H]-H_2O release assay was verified in our laboratory by the product extraction assay (38).

Kinetic determinations. The inhibition kinetic studies were performed using unfractionated white button mushroom extract. The concentration range of [1β-^{3}H]A ranged from 10 to 200 nmol/L. The rate of the reaction was previously established in our laboratory to be linear under these conditions (31–33,40,41). Analyses were performed in triplicate and the data are expressed as the mean ± SD.

Aromatase "in cell" activity in MCF7aro cells. MCF7aro cells are androgen-responsive through aromatase-dependent conversion of testosterone to estrogen. MCF7aro cells (2×10^5 cell/well) were cultured in 6-well plates in Eagles's minimum essential medium (MEM) with Earle's salts (Irvine Scientific, Santa Ana, CA) with 1 mmol/L pyruvate, 2 mmol/L glutamine (Irvine Scientific) and 10% heat-inactivated fetal calf serum (ΔFCS) (Omega Scientific, Tarzana, CA) overnight. The following day the medium was changed to phenol red minus MEM (ϕ^-MEM) (Irvine Scientific) with 10% charcoal dextran–treated heat-inactivated serum (cdΔFCS) for 24 h. The medium was removed and fresh cdΔFCS medium containing the additives was introduced. Cells were cultured in cdΔFCS (5 mL/well) alone (control), with 2.5, 5 or 10 μL of 10X mushroom extract reconstituted in culture medium ($10X_{CM}$)/mL cdΔFCS or 4-OHA (100 μmol/L) as a control inhibitor. After the cells were cultured for 48 h, the medium was removed and the cells were washed twice with 5 mL PBS. The "in cell" aromatase assay was performed with [1β-^{3}H]A in ϕ^-MEM without calf serum for 1 h as described (42). The supernatant was removed and extracted as previously described (42) with 1 mL chloroform to remove unreacted [1β-^{3}H]A. The aqueous layer containing the aromatase product, [^{3}H]-H_2O, was extracted with dextran-treated charcoal. An aliquot of the supernatant was taken for radioactive determination. The cells were solubilized with 1 mL of 0.5 mol/L NaOH. Protein concentration was determined by the Bradford assay (43). Aromatase activity was expressed as pmol [^{3}H]-H_2O produced/(mg protein·h). Analyses were performed in triplicate and the data are expressed as the mean ± SD.

Cell proliferation in the presence or absence of white button mushroom extract. MCF7aro cells were cultured in 6-well plates under the conditions described above for the "in cell" aromatase assay with or without mushroom extract for 48 h. Cells were washed twice with 5 mL PBS and solubilized with 1 mL of 0.5 mol/L NaOH. Cellular proliferation was determined by quantification of protein synthesis by the Bradford assay (43). The data are reported as mean mg protein/L ± SD. The use of protein measurements for aromatase-mediated cell proliferation studies was previously confirmed by cell count analyses (42).

Fractionation of mushroom extract. Mushroom extract (5 mL of $10X_{H2O}$) was fractionated using a Sep-Pak C18 solid phase cartridge (Waters, Milford, MA) by sequential elution with a step gradient solvent system ranging from 5 to 90% acetonitrile/H_20. Fractions were lyophilized and reconstituted in 500 μL water for fractions between 5 and 60% acetonitrile or in 10% dimethyl sulfoxide:H_2O for the fractions collected from 70 to 100% acetonitrile/H_20. Solvent controls were assayed. Incremental fractions were assayed for inhibition of aromatase in the placental microsome assay as described above for a 10-min incubation time. Dose-response quantification was performed using three volumes (10, 25 and 50 μL) in triplicate.

Statistical analysis. The data were analyzed by ANOVA or student's *t* test for the "in cell" aromatase activity and for total protein in the presence or absence of testosterone. Differences were considered significant when $P < 0.05$.

RESULTS

Inhibition of aromatase by vegetable extracts. Recent data from our laboratory demonstrated that phytochemicals in grape juice could suppress aromatase activity (34). No data are available on the effect of specific vegetables on aromatase activity. Seven common vegetables were selected to test the hypothesis that vegetables may contain inhibitors of aromatase activity. The aqueous extraction method for natural compounds has been described (35). This method was selected to avoid toxic solvents that would require removal before use in subsequent "in cell" assays. To enhance the recovery of natural compounds from biological materials, heat was used to break up the compartmentalization found in these materials (35). In addition, heat inactivates plant enzymes that could potentially interfere with the aromatase enzyme assay. Preparations of heat-stable compounds are also valuable for later biological applications in animal and human trials in which sterility is

[5] Abbreviations used: [1β-^{3}H]A, 1β-^{3}H-androstenedione; ER^+, estrogen receptor positive; ΔFCS, heat-inactivated fetal calf serum; cdΔFCS, charcoal dextran–treated heat-inactivated fetal calf serum; MCF7aro, MCF7 cells transfected with human aromatase; MEM, Eagle's minimum essential medium; ϕ^-MEM, phenol red minus Eagle's minimum essential medium; 4-OHA, 4-hydroxyandrostenedione.

essential. Of the seven vegetable extracts tested, the extract from white button mushroom (*Agaricus bisporus*) was the most effective in inhibiting human placental aromatase (**Fig. 1**). A 50% inhibition of aromatase activity was achieved with 50 μL of $7.5X_{H2O}$ mushroom extract. The active component in the mushroom extract appeared to be water soluble and heat stable. Of the other extracts evaluated, celery had a modest inhibitory effect. Extracts prepared from green onion, carrot, bell pepper, broccoli and spinach did not inhibit aromatase under these experimental conditions.

Aromatase inhibition by extracts from different varieties of mushrooms. To determine whether aromatase inhibition was unique to the white button mushroom, ten additional varieties of mushrooms were evaluated, i.e., woodear (*Auricularia* spp.), crimini (*Agaricus bisporus*), white button mushroom (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), Italian brown (*Pleurotus* spp.), enoki (*Flammulina velutipes*), baby button mushrooms (*Agaricus bisporus*), stuffing (*Agaricus bisporus*), shiitake (*Lentinula edodes*), chanterelle (*Cantharellus* spp.) and portobello (*Agaricus bisporus*) (**Fig. 2**). The studies identified that the stuffing mushroom had the most potent inhibitory effect against aromatase activity. The shiitake, white button mushroom, portabello, crimini and baby button mushrooms also demonstrated significant antiaromatase effects. These findings suggest that a number of varieties of mushroom possess inhibitory effects on aromatase activity in an in vitro assay. All subsequent studies were performed using heat-stable extracts from the white button mushroom.

Aromatase inhibition kinetic analysis with white button mushroom extract. The double reciprocal plot of aromatase inhibition with respect to androstenedione by white button mushroom extract is shown in **Figure 3**. The mixed-type inhibition kinetic profile for the whole-mushroom extract suggested the presence of one or more inhibitors in the extract and/or more than one inhibitory mechanism.

Characterization of white button mushroom extract. Fractionation of the crude white button mushroom extract was performed using a step gradient on Sep-Pak C18 solid phase extraction cartridges (**Fig. 4**). Dose-response experiments were performed using the human placental microsome assay. The maximum inhibition was identified in the 15% elution fraction. These results indicate the presence of more than one antiaromatase chemical in the white button mushroom extract. Scale-up isolation, purification and structural characterization of these antiaromatase chemicals are currently underway in our laboratory.

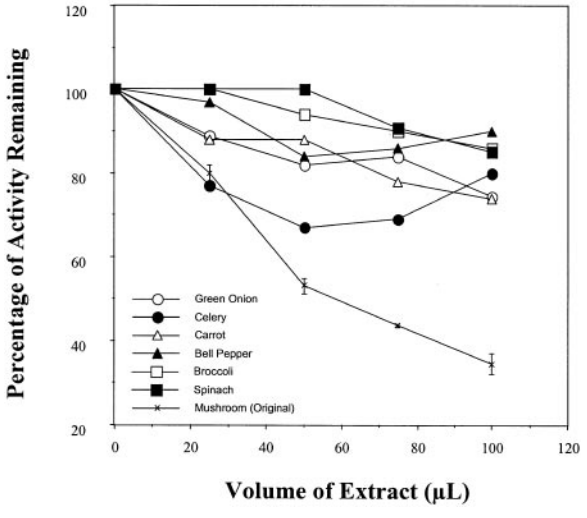

FIGURE 1 Inhibition of aromatase by vegetable extracts. In vitro human placental aromatase assay was performed in the presence of increasing amounts of the vegetable extracts ($7.5X_{H2O}$). The aromatase activity of untreated microsomes was set at 100%. The measurements were performed in triplicate. For simplicity, only the standard error bars for the inhibition curve of white button mushrooms are shown. The aromatase activity of the untreated control and those of samples that were treated with the white button mushroom extract differed, $P < 0.0002$.

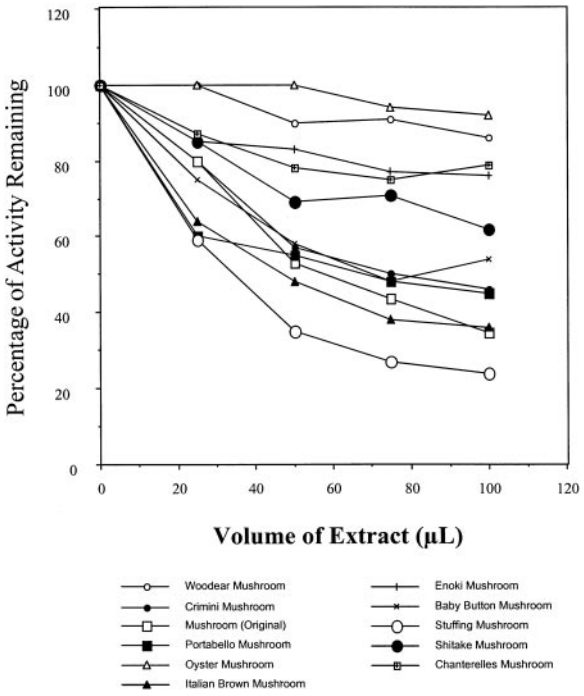

FIGURE 2 Characterization of inhibition of aromatase by aqueous extracts from different varieties of mushrooms. The aromatase activity in the presence of heat stable extracts from eleven varieties of mushrooms, woodear (*Auricularia* spp.), crimini (*Agaricus bisporus*), white button mushroom (*Agaricus bisporus*), oyster (*Pleurotus ostreatus*), Italian brown (*Pleurotus* spp.), enoki (*Flammulina velutipes*), baby button mushrooms (*Agaricus bisporus*), stuffing (*Agaricus bisporus*), shiitake (*Lentinula edodes*), chanterelle (*Cantharellus* spp.), portobello (*Agaricus bisporus*) was performed as described for Figure 1.

"In cell" aromatase assay. The "in cell" aromatase assay was performed to determine the feasibility of introducing the extract in a whole-cell system. The dose-response effect of white button mushroom extract on "in cell" aromatase activity was determined in MCF7aro cells cultured in the presence or absence of testosterone (**Fig. 5**). The cells were cultured without mushroom extract or in the presence of increasing concentrations of mushroom extract. The aromatase inhibitor, 4-OHA, was used as the known inhibitor for a positive inhibitory control. Mushroom extract inhibited aromatase activity in a dose-dependent manner with all three concentrations of white button mushroom extract evaluated. In the presence of testosterone, an aromatase substrate, the basal aromatase activity was increased. This testosterone-dependent increase in aromatase activity remained in the presence of the lowest concentration (2.5 μL) of mushroom extract. When the mushroom extract concentration was increased, there was a proportional decrease in the testosterone-dependent aromatase activity with the intermediate dose of mushroom extract (5 μL). In the presence of the highest concentration of mushroom extract (10 μL), the aromatase activity was inhib-

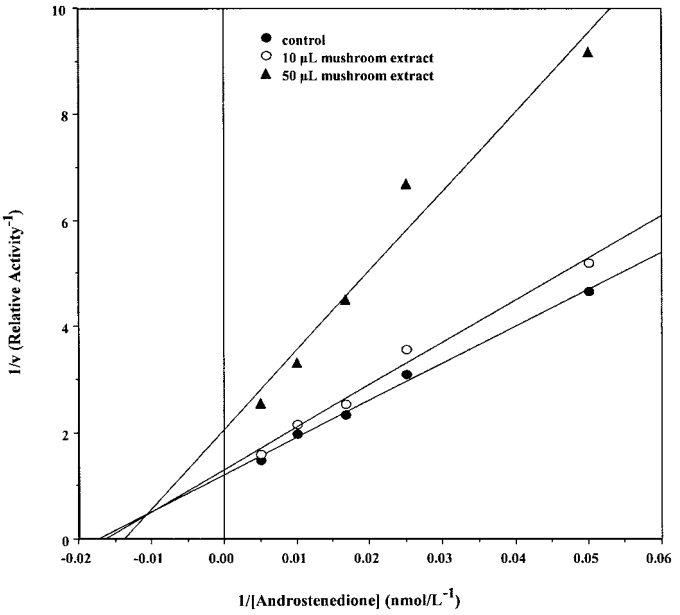

FIGURE 3 Aromatase inhibition kinetic profile by white button mushroom (*Agaricus bisporus*) aqueous extract. The enzyme assay was performed in the presence of 0, 20 and 50 μL of white button mushroom extract reconstituted in H_2O ($10X_{H2O}$) per 500 μL reaction mixture and with increasing concentration of [^{3}H]-androstenedione as described in Materials and Methods. The concentration range of [^{3}H]-androstenedione was 10–200 nmol/L. The assay was performed in triplicate.

ited to the same degree whether the cells were cultured in the presence or absence of testosterone. This suggests that the testosterone may compete with one or more of the putative components of mushroom extract, producing a protective effect.

Effect of mushroom extract on cell proliferation. MCF7aro cells were transfected with the human aromatase enzyme. These cells are ER$^+$ and demonstrate increased cell proliferation in the presence of testosterone. Cell proliferation was determined by measuring total cellular protein in the presence or absence of the mushroom extract as has been previously described (42). The dose-response effect of mushroom extract on total protein expression in MCF7aro cells cultured in the presence or absence of testosterone is shown in **Figure 6**. The aromatase inhibitor, 4-OHA, was used as the known inhibitor. Cells grown in the presence of testosterone demonstrated increased proliferation. This is the expected response in MCF7aro cells that are transfected with aromatase and are ER$^+$. Exposure to low doses of mushroom extract did not decrease the total amount of protein below the control conditions at 48 h. The highest dose of mushroom extract, however, decreased the advantage gained by the addition of testosterone to a level similar to that seen with 4-OHA. These findings suggest that the inhibitory effect of white button mushroom extract is through a specific antiaromatase action, not a cytotoxic effect.

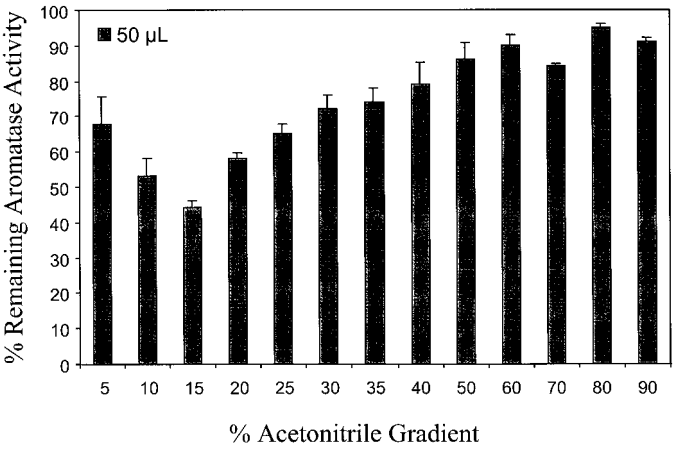

FIGURE 4 Step gradient fractionation of white button (*Agaricus bisporus*) mushroom extract. Fractionation of 5 mL of the lyophilized and reconstituted crude white button mushroom extract ($10X_{H2O}$) was performed using a step gradient on Sep-Pak C18 solid phase cartridges. Dose response data were quantified using the human placental microsome assay. The assay was done in triplicate for each dose at each eluted fraction. The data are expressed as the percentage of activity remaining compared with the untreated control, which is expressed as 100%.

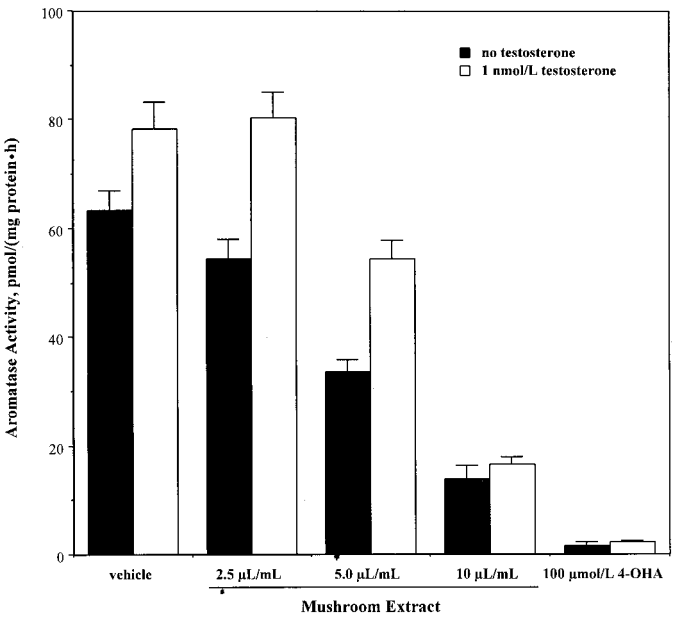

FIGURE 5 Aromatase "in cell" assay in MCF7 cells transfected with human aromatase (MCF7aro) in the presence of white button (*Agaricus bisporus*) mushroom extract with and without testosterone. The dose-response effect of mushroom extract on "in cell" aromatase activity was evaluated in MCF7aro cells (2×10^5 cells/well) cultured in six-well plates with and without 1 nmol/L testosterone. The cells were cultured without mushroom extract or with 2.5, 5 or 10 μL lyophilized mushroom extract reconstituted in culture medium ($10X_{CM}$)/mL of cell culture medium (5 mL/well). The aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA), was used as the inhibitor control. The assay was done in triplicate and the results are expressed as mean ± SD. Aromatase activity differed among groups for cells grown in the absence ($P < 0.001$) and in the presence ($P < 0.001$) of testosterone. The vehicle control without testosterone differed from the testosterone control ($P = 0.0122$). The samples that were treated with mushroom extract at 2.5 μL with and without testosterone differed ($P = 0.002$). The samples that were treated with mushroom extract at 5 μL with and without testosterone tended to differ ($P = 0.06$).

DISCUSSION

In the search for new approaches to breast cancer chemoprevention, known pharmaceutical agents have been tested for their potential beneficial effects. Chemoprevention aims to

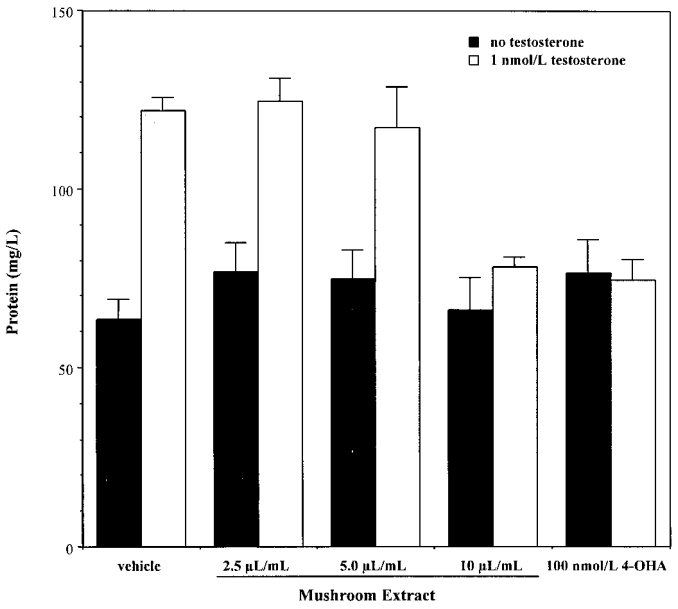

FIGURE 6 Effect of white button (*Agaricus bisporus*) mushroom extract on cell proliferation of MCF7 cells transfected with human aromatase (MCF7aro) in the presence and absence of testosterone. The dose response effect of mushroom extract on total protein expression was determined in MCF7aro cells (2×10^5 cells/well) cultured in six-well plates with and without testosterone. Cells were cultured and protein determined as described in Materials and Methods. 4-Hydroxyandrostenedione (4-OHA) was used as the aromatase inhibitor control. The experiments were performed in triplicate and the results are expressed as mean ± SD. Total protein levels differed among groups of cells grown in the presence of testosterone ($P < 0.001$). Total protein levels in the vehicle control without testosterone differed from the testosterone control ($P = 0.0001$).

modulate specific steps in the neoplastic process including prevention of DNA damage by free radicals, inhibition of cellular proliferation and promotion of cellular differentiation (44). A major strategy for breast cancer chemoprevention is directed at abolishing the estrogen effects. The in situ reduction of estradiol synthesis by inhibition of aromatase could reduce estrogen-dependent cellular proliferation and estrogen conversion to genotoxic metabolites (12).

The present study was undertaken to screen and evaluate a number of vegetables as potential natural sources of aromatase inhibitors. Heat-stable, water-soluble extracts were prepared. A recent study reported that serum concentrations of α-carotene and lutein were higher when raw vegetable juice was consumed than when raw vegetables or cooked vegetables were consumed (45). The current approach was undertaken to maximize extraction of small molecules but avoid toxic solvents (35). Using an in vitro human placental microsome aromatase assay, the white button mushroom was found to be a potent inhibitor of aromatase. The assay of aromatase activity in human placental microsomes in the presence of a variety of compounds is an established model to measure relative inhibitory effects of a series of chemopreventive compounds (46). We further confirmed the biological activity of white button mushroom in an aromatase-transfected breast cancer cell line to confirm the inhibitory effect in an "in cell" model. The white button, shiitake, portabello, crimini and baby button mushroom varieties demonstrated the ability to inhibit aromatase activity in an in vitro assay. The work presented here focused on the white button mushroom because it demonstrated potent inhibition and is easily available during all seasons. Furthermore, the white button mushroom is less costly than other varieties of mushrooms, making it more readily purchased by the average consumer.

MCF7aro cells are estrogen dependent for growth. Transfection with human aromatase provides them with the ability to synthesize estrogens and leads to enhanced growth. Determination of the effect of white button mushroom extract on cellular proliferation has revealed that the heat-stable extract suppresses only the androgen-induced cellular proliferation as determined by total cell protein. The total protein determination in cells without androgen did not demonstrate a decrease at the three doses of mushroom extract evaluation, indicating that the extract was not toxic. The use of protein measurements for aromatase-mediated cell proliferation studies has been previously confirmed by cell count analyses (42).

Mushrooms have been recognized for their edible and functional properties for centuries (47). Edible mushrooms have beneficial effects on health and in the treatment of disease through their immunomodulatory, antineoplastic and lipid-reducing properties (47). The shiitake mushroom has served as the model for investigating functional mushrooms and isolating pure compounds for pharmaceutical use. Water extracts of the shiitake mushroom prevent tumor growth in mice (48). We have demonstrated that a water-soluble extract from the white button mushroom can suppress aromatase activity.

The data from our study suggest that the white button mushroom may have potential therapeutic benefit as a functional food by reducing the activity of aromatase, the putative enzyme for converting androgens to estrogens. These studies have been performed using the crude extract to avoid potential changes in efficacy by the purification process and to maintain a close resemblance to the composition as a food source. The whole extract demonstrates efficacy in an "in cell" assay system producing aromatase inhibition without toxic side effects. The limitation of this approach is that this extract contains a spectrum of compounds that are further metabolized and may produce potent intermediary molecules that participate in alternative mechanisms.

Future work will focus on isolation, purification and characterization of the components. Animal studies using time-course and dose-response data are being designed to test the whole extract and compare it to the most potent purified components. Specific site-directed mutants of the aromatase binding pocket will serve to characterize the binding characteristics of the purified components and compare the inhibitory activity and binding capacity of the mushroom extract with known aromatase inhibitors (31,32,41,49,50). The white button mushroom demonstrates the ability to inhibit aromatase activity in vitro and in a cell culture system, but like other mushroom varieties, it may contain a number of other bioactive compounds with diverse biological activity. The purified compound(s) may be very different from the whole mushroom in their inhibitory activity on aromatase. The inevitable questions that follow are whether eating the whole mushroom has preventative or therapeutic value, and if so, how much mushroom should be consumed to achieve a beneficial effect. Future studies should answer some of these questions and provide guidelines for incorporation of mushrooms and/or their extracts into the diet for their "nutraceutical" properties.

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