Available online at www.sciencedirect.com
ScienceDirect
Food and Chemical Toxicology 46 (2008) 310–320
www.elsevier.com/locate/foodchemtox

A dietary supplement for female sexual dysfunction, Avlimil, stimulates the growth of estrogen-dependent breast tumors (MCF-7) implanted in ovariectomized athymic nude mice

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Received 24 March 2007; accepted 10 August 2007

Abstract

Avlimil, a dietary supplement advertised to ameliorate female sexual dysfunction, is a mixture of eleven herbal components, and some herbal constituents of Avlimil (including black cohosh, licorice, red raspberry, red clover and kudzu) contain phenolic compounds, which are suggested to have estrogenic, anti-estrogenic, or androgenic potential for relieving menopausal symptoms. We hypothesize that Avlimil could modulate the growth of estrogen receptor positive human breast cancer (MCF-7) cells *in vitro* and *in vivo*. A dimethylsulfoxide (DMSO) extract of Avlimil (0.001–100 μg Avlimil powder equivalents/mL media) was tested for its estrogenic and anti-estrogenic effects on the growth of MCF-7 cells *in vitro*. We observed that the DMSO extract of Avlimil at low concentrations (0.1–50 μg/mL media) dose-dependently increased MCF-7 cell proliferation *in vitro*, and Avlimil DMSO extract at 100 μg/mL inhibited the growth of MCF-7 cells *in vitro*. Avlimil and some constituents (black cohosh and licorice roots) of Avlimil were fractionated by using sequential solvent extraction (hexane, ethyl acetate, and methanol) and the activities of the fractions were monitored by effects on the growth of MCF-7 cells. Depending on dosage (0.1–100 μg/mL media) both stimulatory and inhibitory effects of the extracts on the growth of MCF-7 cells were observed. The effect of dietary Avlimil at dosages approximating human intake was evaluated using ovariectomized mice implanted with MCF-7 cells. Animals were fed diets containing 500 ppm or 1000 ppm Avlimil for 16 weeks. Dietary Avlimil at 500 ppm stimulated MCF-7 tumors, but Avlimil at 1000 ppm had no apparent effect on the growth of MCF-7 tumors. The observation of stimulated tumor growth in the absence of uterine wet weight gains suggest that estrogenic/anti-estrogenic effects of Avlimil we observed may be dosage- and target tissue-specific and that Avlimil may not be safe for women with estrogen-dependent breast cancer. The different biological effects of fractionated Avlimil components and the different concentration dependencies warrant further compound identification and dose-response studies, especially at recommended intake levels that could have estrogenic effects in women.

Keywords: Avlimil; Breast cancer; MCF-7; Black cohosh root; Licorice root; Isoflavones

Abbreviations: AIN93G, American Institute of Nutrition 93 growth semi-purified diet; BC, black cohosh; BCS, bovine calf serum; CD-BCS, charcoal-dextran stripped BCS; DMSO, dimethylsuloxide; E_2, 17β-estradiol; ER, estrogen receptor; HRT, hormone replacement therapy; LC, licorice root; MCF-7, Michigan Cancer Foundation-7; MEM, minimal essential media; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); qRT-PCR, reverse transcription polymerase chain reaction; SE, standard error.

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0278-6915/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.fct.2007.08.006

1. Introduction

For women aged 40–55 breast cancer is the second leading cause of cancer death in the United States (Cancer.org, 2005a,b). However, most breast cancer cases (~77%) occur in postmenopausal women and most are estrogen receptor (ER)-positive (Harlan et al., 2002). Many breast cancer survivors experience sexual dysfunction (McKee and Schover, 2001), estimated at approximately 50% of long-term survivors (Ganz et al., 1998). Avlimil (also known as *Salvia rubus*) (Warner Health Care, 2003a,b), is the leading non-prescription dietary supplement for female sexual health, and has been on the market since January, 2003. In 1 year, the number of Avlimil customers reached 500,000 with the majority enrolled in a long-term preferred customer plan allowing for chronic exposure to these potentially estrogenic botanicals (Avlimil One Year Later, 2004). Avlimil is targeted to approximately 50 million women who have sexual problems for different reasons, including hormonal changes, menopause or aging processes where is the reference (CBS news, 2003 ['women who have undergone hormonal fluctuations due to menopause or childbirth may find Avlimil helpful in restoring a sense of sexual well-being, without the use of hormone replacement therapy (HRT) or other prescription drugs' Warner Health Care (2003a,b)]. The major herbal components in Avlimil are *Salvia officinalis* (sage leaf), *Rubus idaeus* (red raspberry leaf), *Pueraria montana* (kudzu root extract), *Trifolium pratense* (red clover extract), *Capsicum annuum* (capsicum pepper), *Glycyrrhiza glabra* (licorice root), *Morella cerifera* (bayberry fruit), *Turnera diffusa* (damiana leaf), *Valeriana officinalis* (valeriana root), *Zingiber officinale* (ginger root), and *Actaea racemosa* (black cohosh root). The flavones (apigenin, hispidulin, cirsimaritin) and abietane diterpenes from *S. officinalis* (sage leaf) act as benzodiazepine receptor-active components (Kavvadias et al., 2003). Quinones (roylanone, 7α-oxyroylanone, and 7α-acetoxyroylanone) from *S. officinalis* have also been reported as bioactive compounds (antimicrobial and anti-inflammatory effects) (Spiridonov et al., 2003). *R. idaeus* (Red raspberry leaf) contains a variety of phenolic compounds and total phenolic contents for leaves are expressed as milligram of gallic acid equivalent per gram fresh weight (Wang and Lin, 2000), and its antioxidant effect has been documented (de Ancos et al., 2000; Kalt et al., 1999; Mullen et al., 2002; Wang and Lin, 2000). The presence of isoflavones in Avlimil was indicated by the inclusion of red clover (Biochanin A and formonetin) and kudzu (puerarin), and their estrogen-like activities have been documented (Boue et al., 2003; Burdette et al., 2002). Although Avlimil does not contain estradiol, some plant materials [black cohosh root (*Cimicifuga racemosa*) and licorice root (*G. glabra*)], contain phenolic or terpenoid components that may have estrogenic and/or anti-estrogenic actions (Zava et al., 1998), and may modulate breast cancer in postmenopausal women. No information regarding consumption of Avlimil and possible safety issues, including estrogen-dependent breast cancer, is available at the present time. It is also possible that this herbal mixture may show various biological effects at different dosages. Without a better understanding of its biological effect it is not possible to generalize that the use of this product is safe for postmenopausal women with breast cancer. In this study, we evaluated the potential estrogenic effects of Avlimil using well-described *in vitro* and *in vivo* assays to address this important women's health concern.

2. Materials and methods

2.1. Chemicals and reagents

Avlimil was purchased from Warner Health Care, Inc. (Wallingford, CT). Minimal Essential Medium (MEM, without gentamicin, with glutamine) and phenol red-free MEM was purchased from the Media Facility at the University of Illinois at Urbana-Champaign. Bovine calf serum (BCS) was purchased from Hyclone (Logan, UT). Penicillin/streptomycin and trypsin/EDTA were purchased from Invitrogen (Houston, TX). Laboratory animal diet and dietary components were purchased from Dyets (Bethlehem, PA). Reagents for qRT-PCR were purchased from PE Applied Biosystems (Foster City, CA), Synthegen (Houston, TX) and Invitrogen (Carlsbad, CA).

2.2. Maintenance of human breast cancer cells

MCF-7 cells are estrogen-dependent tumor cells isolated from a postmenopausal woman (Soule et al., 1973). MCF-7 cells were maintained in MEM supplemented with 5% BCS, 1% penicillin (100 U/mL)/streptomycin (100 μg/mL), and 1 nM 17β-estradiol (E_2). MCF-7 cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air as a monolayer culture in plastic culture plates (100 mm dia.). Seven days before the cell proliferation assay or the injection of MCF-7 cells into athymic mice, the media was switched to phenol red-free MEM containing 5% charcoal dextran stripped (CD)-BCS (Ju et al., 2001) and 1% pen/strep. MCF-7 in passages between 350 and 370, were used for cell assay and tumor growth study.

2.3. Preparation of extracts

2.3.1. DMSO Avlimil extract

To characterize the complex mixture of botanical products in Avlimil, we used a simple fractionation method that minimizes degradation and allows for systematic evaluation of the extracts using a cell growth assay. The purple film coat of Avlimil tablet was removed using a razor blade. The naked tablet was ground to a fine powder using a mortar and pestle. The Avlimil powder was mixed in DMSO (1 g in 10 mL) in a centrifuge tube, vortexed vigorously, incubated at 37 °C for 30 min, and centrifuged for 10 min at 1000 rpm at 24 °C. The supernatant portion, containing the equivalent of 100 μg of the original Avlimil powder/μL, was recovered, filter-sterilized (0.45 μm), aliquoted, stored at −20 °C, and used for *in vitro* cell assay. The insoluble material remaining was discarded.

Bioactive material bound to the filter and the insoluble materials that may have activity were not assayed due to inherent technical problems associated with this type of experimentation.

2.3.2. Sequential hexane, ethyl acetate and methanol Avlimil extracts

The Avlimil powder was mixed with hexane (1 g/mL) in a glass tube, vortexed vigorously, incubated at 37 °C for 30 min, and centrifuged for 10 min at 1000 rpm at 24 °C. The supernatant was decanted and dried using nitrogen gas, reconstituted with DMSO as above (100 μg Avlimil powder equivalents/μL), filter-sterilized, aliquoted, stored at −20 °C, and used for *in vitro* cell assays. The resulting solids portion was air-dried, and mixed with ethyl acetate (1 g/mL) in a glass tube, vortexed vigorously,