

Case Report

Prolonged stabilization of platinum-resistant ovarian cancer in a single patient consuming a fermented soy therapy

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Abstract

Background. Women with ovarian cancer who experience disease progression during or within 6 months of first-line treatment with platinum-based anticancer drugs are considered to have platinum-resistant tumors. These patients have an unfavorable prognosis, and they frequently seek complementary and alternative therapies (CAM). Historically, this represents an understudied and underreported component of ovarian cancer treatment.

Case. This report describes the case of a woman with rapidly progressive, platinum-resistant ovarian cancer. Upon initiating self-directed treatment with Haelan951[®], a commercially available fermented soy beverage, she entered into a phase of prolonged disease stabilization including improvement in the serum tumor marker CA-125.

Conclusion. Fermented soy products are known to contain high concentrations of the isoflavone, genistein, and other compounds that exhibit anticancer activity in preclinical models. This case report supports the prospective evaluation of alternative therapies such as these in patients with platinum-refractory ovarian cancer.

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Introduction

Currently, the standard therapy for women with newly diagnosed ovarian cancer is carboplatin typically delivered with paclitaxel [1]. Women who experience progressive disease during primary chemotherapy or cancer progression within 6 months of completing primary treatment are considered to have platinum-refractory or -resistant tumors and have an unfavorable prognosis [2]. While several chemotherapeutic agents demonstrate modest activity in this clinical setting, responses are typically brief in duration [3]. Many of these patients consider participating in clinical trials of investigational new drugs or

novel approaches of approved anticancer agents, such as alternative administration schedules and drug combinations. In addition, it has been reported that patients with platinum-resistant disease frequently use complementary and alternative therapies (CAM), often without the knowledge of their caregivers [4,5]. Some of the most readily available, ingestible therapies include mistletoe, shark cartilage, various soy teas, and cat's claw. Patients who consider using CAM tend to be well-educated, young women of higher socioeconomic status [4,5]. A survey of cancer patients and care providers demonstrated that providers estimated 40% of their patients used CAM. However, self-reporting from patients demonstrated that true usage rate was as high as 80% [4]. The therapeutic benefit of alternative therapies used by cancer patients is largely unknown as reports of responses are highly anecdotal, and they have rarely been the objective of prospective clinical trials suitable for publication in peer-reviewed medical journals.

This report highlights a single case of a woman whose self-directed use of Haelan 951, a commercially available fermen-

Abbreviations: EGF, epidermal growth factor; GOG, Gynecological Oncology Group; PIP, phosphatidyl inositol,4 phosphate-kinase; TAH/BSO, total abdominal hysterectomy and bilateral salpingo-oophorectomy; TGFβ1, tissue growth factor-beta-1; LC/MSD, liquid chromatography with mass spectrometric detection.

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ted soy beverage, was associated with a prolonged period of disease stabilization as documented by serial radiographic tumor measurements and serum CA-125 levels. The evidence of tumor response in a woman with rapidly progressive and platinum-resistant disease is particularly notable observation. Pharmacokinetic studies of both the product and serum drawn from the patient shortly after ingestion support the hypothesis that high concentrations of isoflavones may have provided an antitumor effect.

Case report

This 56-year-old woman presented in March 2002 to her primary care physician with a 1-month history of fatigue, abdominal discomfort, constipation, and bloating. Physical examination and radiographic diagnosis by computed tomography revealed an omental and adnexal mass with ascites and a serum CA-125 level of 2323 IU/mL. On April 5, 2002, she underwent exploratory laparotomy, TAH/BSO, total gastrosigmoidectomy, tumor debulking, removal of pelvic peritoneum, appendectomy, and resection of her abdominal wall mass. Pathologic evaluation of the tumor revealed a papillary serous ovarian carcinoma. Immunohistochemical staining showed the tumor was negative for the α ER receptor and the α and β PR receptor. Her CA-125 concentration decreased to 756 IU/mL after surgery.

The patient initiated chemotherapy on a clinical trial involving three sequential chemotherapy doublets of gemcitabine/carboplatin (cycles 1–3), paclitaxel/carboplatin (cycles 4–6), and adriamycin/topotecan (cycles 7–9). At the conclusion of nine cycles of primary chemotherapy, including six cycles of platinum-based therapy, her serum CA-125 had not normalized (final value 50), and second look laparoscopy at the conclusion of her chemotherapy in November 2002 documented gross multifocal peritoneal disease. She received no therapy from November 2002 until March 2003. During that period of time, her serum CA-125 increased to 1295 IU/mL coupled with onset of progressive constipation. She was enrolled in a phase II trial of the investigational cytotoxic drug ecteinascidin 743 in April 2003 and experienced a partial response that persisted for a 6-month duration. She was removed from the study in November 2003 due to progressive disease documented by an abdominal CT demonstrating new perihepatic disease and modest retroperitoneal nodes.

Subsequently, she self-initiated the consumption of an herbal tea (Pau d'Arco; Pau-D-Arco.com, Lake Elsinore, CA) during which time her serum CA-125 increased from a level of 171 IU/mL upon removal from the clinical trial to 477 IU/mL 2 months later. She declined further treatment with conventional therapies, and in March of 2004, she began consuming approximately 4 oz of a fermented soy beverage, Haelan 951 (Haelan Products, Inc, Woodinville, WA) once a day under her own direction. She was examined at MGH on April 6, 2004, after approximately 6 weeks of drinking Haelan 951. She was evaluated and found to have no symptoms with a GOG performance score of 0, normal exam, and a serum CA-125 concentration that declined to 311 IU/mL (see Fig. 1). A CT

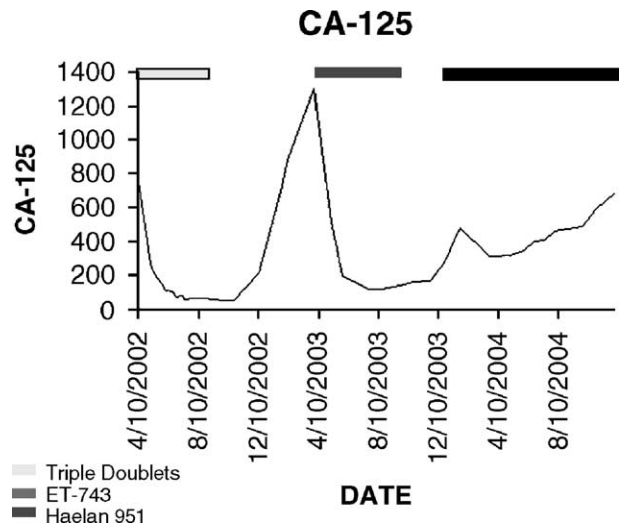


Fig. 1. Plot of CA-125 from initial diagnosis (April 2002) through late 2004. The patient was off chemotherapy from late 2002 through March 2003 and again from November 2003 through December 2004. She began Haelan 951 in late February 2004.

scan obtained in March 2004 showed stable metastatic disease involving the peritoneum and paraaortic/peridiaphragmatic lymph nodes. CT scans acquired in August, October, and December of 2004 all showed minimal progression of retroperitoneal nodes. In November of 2004, after obtaining approval from the Institutional Review Board and signed informed consent from the patient, blood samples were collected from the patient for determining the concentration of isoflavones in plasma immediately before and 1 h after the ingestion of Haelan 951. She continued to consume the Haelan 951 for 9 months, until December 14, 2004, when she agreed to resume treatment with carboplatin. At that time, she had evidence of a rising CA-125, progressive retroperitoneal adenopathy, and the development of worsening constipation. The patient had an excellent clinical response to carboplatin while continuing to take Haelan 951.

Isoflavone determinations

Experimental procedures

Blood specimens for isoflavone determinations were drawn from a peripheral vein and collected in tubes containing sodium heparin anticoagulant. Samples were obtained 8 h after oral ingestion of a 4-oz fluid dose of Haelan 951 and 1 h after taking a subsequent dosage. Sample tubes were promptly centrifuged, and the plasma was separated for storage at -70°C until assayed.

The aglycone isoflavones genistein and daidzein were measured by high-performance liquid chromatography with mass spectrometric detection (LC/MSD) as previously described [6], with minor modifications. An Agilent Technologies (Palo Alto, CA) 1100 Series LC/MSD system consisting of a quaternary pump, autosampler, and a model XCT ion trap mass spectrometer was used. Reference samples of the isoflavones with a minimum purity of 98% were obtained from commercial sources and used directly for preparing calibration curves

Table 1
Assayed concentration and estimated dosage of isoflavones in Haelan 951

Compound	Assayed concentration (μM)		Estimated daily dosage (mg) ^a	
	Free (percent of total)	Total	Free	Total
Daidzein	127 (26)	500	3.8	15.1
Genistein	46 (5)	955	1.5	30.5

^a Amounts in 4-oz fluid of Haelan 951.

without further purification. Calibration standards were prepared by adding daidzein and genistein to human plasma at concentrations ranging from 1 to 100 ng/mL.

Samples of Haelan 951 Platinum Formula (batch no. 4031123; Haelan Products, Inc., Woodinville, WA) and plasma were directly prepared for LC/MS analysis, as described below, to determine concentrations of the aglycone isoflavones. Total concentrations of genistein and daidzein in Haelan 951 were determined by preliminary enzymatic hydrolysis of the glycoside conjugates [7]. Briefly, 1 mL of Haelan 951 was freeze dried and extracted with 5 mL of methanol–water (80:20, v/v) by sonicating for 20 min and allowing the mixture to stand overnight before removing insoluble matter by filtration. After evaporating the solvent from the filtrate, 40 units of cellulase from *Aspergillus niger* (Sigma-Aldrich Co., St. Louis, MO) in 2 mL of 0.1 M ammonium acetate buffer, pH 5.0, was added to the extract. The sample was sonicated and incubated overnight at 37°C. To determine the concentration of isoflavone glucuronides, plasma (50 μL) was incubated together with 50 μL of a 10,000 unit/mL suspension of β -glucuronidase (Type B-1 from bovine liver; Sigma-Aldrich) in 0.1 M ammonium acetate buffer, pH 5.0, for 2 h at 37°C (ref).

Samples (100 μL) of Haelan 951 and plasma, either without pretreatment or after enzymatic hydrolysis, were extracted with 3 mL of *tert*-butyl methyl ether after adding 10 μL of 1 $\mu\text{g}/\text{mL}$ solution of 7-hydroxyflavone in dimethylsulfoxide as an internal standard. The organic phase was removed, the solvent evaporated, and the extract reconstituted with 150 μL of methanol–acetonitrile–10 mM ammonium formate (20:20:60, v/v/v). The sample solution (100 μL) was loaded onto a 15-cm \times 4.6-mm Luna 5- μm C18 HPLC column (Phenomenex, Torrance, CA), preceded by a 4 \times 3-mm precolumn of the same stationary phase and a 0.5- μm inline filter. The column was eluted at ambient temperature using a ternary mobile phase, delivered at 1.0 mL/min, composed of methanol–acetonitrile–10 mM ammonium formate at proportions that were changed from 20%:20%:60% at the beginning of the run to 45%:45%:10% linearly over 13 min. The final proportion was maintained until the run concluded 4 min later, whereupon the column was allowed to equilibrate with the initial composition of the mobile phase for 5 min before the next injection. Retention times of the compounds were 5.2 min for daidzein, 7.0 min for genistein, and 8.2 min for the internal standard. The mass spectrometer was fitted with an electrospray ionization interface using nitrogen as the drying gas (flow rate, 12 L/min; temperature, 250°C), with a nebulizer pressure of 30 psi and a capillary voltage of 4.5 kV. Tuning parameters of the mass spectrometer were optimized for the detection of genistein.

Quantitation was performed by isolating negative ions corresponding to the deprotonated parent molecules ($[\text{M}-\text{H}]^-$) of daidzein at m/z 253.1, genistein at m/z 269.0 and the internal standard at m/z 237.1, using a mass width of 1 amu, without fragmentation.

Results

Concentrations of daidzein and genistein in Haelan 951 from a bottle that was supplied by the patient, determined in our laboratories both as the aglycone and total compound (i.e., aglycone plus glycoside conjugates) by LC/MS, are given in Table 1. Both isoflavones were present, predominantly as glycoside conjugates, at total concentrations of 500 μM for daidzein and 955 μM for genistein. The results are comparable to analytical data available on the manufacturers website (www.haelanproducts.com/research.htm), indicating that the product contained daidzein and genistein at total concentrations of 724 μM (184 $\mu\text{g}/\text{mL}$) and 844 μM (228 $\mu\text{g}/\text{mL}$), respectively. The daily dose of each compound provided in 4 oz fluid of Haelan 951 is also indicated in Table 1. Concentrations of genistein and daidzein in plasma samples obtained from the patient shortly before and 1 h after ingesting her usual 4-oz fluid dose of Haelan 951 are presented in Table 2. The pretreatment sample was collected approximately 8 h after taking the previous dose. One hour after dosing, the concentration of genistein glucuronide in plasma increased to 501 nM from a pretreatment level of 300 nM, while the daidzein glucuronide concentration increased from 393 to 678 nM. Plasma levels of unconjugated genistein remained below the 3.7 nM lower limit of quantitation of the assay, although the concentration of unconjugated daidzein became measurable 1 h after dosing, achieving a concentration of 17 nM.

Discussion

We present a case of a woman with platinum-resistant ovarian cancer who has had asymptomatic, stable disease for 9 months on Haelan 951. In the two prior periods when the patient was off chemotherapy, she demonstrated a CA-125 doubling time of less than 1 month. She initiated Haelan 951 with a serum CA-125 concentration exceeding 400 IU/mL, which rose to 688 IU/mL over the course of 9 months, corresponding to a doubling time in excess of 1 year. Her clinical course, serum CA-125 levels, and physical and

Table 2
Isoflavone concentrations in plasma samples obtained from the patient before and after ingesting Haelan 951

Sample	Genistein (nM)		Daidzein (nM)	
	Aglycone	Total glucuronide	Aglycone	Total glucuronide
Pretreatment (8 h after prior dose)	<3.7	300	<3.9	393
1 h after dosing	<3.7	501	17	678

radiologic examinations over the past year provide evidence suggestive of meaningful clinical activity of Haelan 951 in this particular patient.

Haelan 951, a fermented soy product delivered in 8-oz bottles, is a pungent, rancid, frothy brown liquid. The use of soybean derived foods in cancer prevention and treatment is based, in part, on the epidemiologic observation that the incidence of breast and prostate cancer is much lower in Southeast Asia where soy consumption is 20-fold higher than in developed countries [8]. Preclinical studies suggest that active compounds in these dietary products are isoflavones. Soy is the major source of isoflavones in foods consumed by humans [9]. Isoflavones occur predominantly as glycoside conjugates in soybeans [7]. The fermentation process hydrolyzes the glycosidic isoflavones genistin and diadzin to the corresponding aglycones, genistein and daidzein, which both have antineoplastic properties *in vitro* [7]. The aglycone form of these compounds is more readily absorbed upon oral administration than the glycoside conjugates [10,11]. Following absorption, isoflavone aglycones are rapidly and extensively metabolized in the liver by glucuronic acid conjugation and sulfation [12].

Analysis of a sample of Haelan 951 obtained from the patient revealed that the unconjugated aglycone forms of the isoflavones represented only 26% of the total daidzein concentration and 5% of the total genistein concentration. Although Haelan 951 is marketed as a “fermented soybean beverage,” the fermentation process does not efficiently convert the glycoside conjugates to the more bioavailable aglycone form of these isoflavones. However, in comparison to common soy-based foods, the total amount of genistein and daidzein in 4 oz fluid of Haelan 951 is approximately 2 times greater than 100 g of tofu and 4 times greater than 100 g of soy milk [9]. Consuming this volume of Haelan 951 on a daily basis produced relatively high plasma concentrations of the two isoflavones, predominantly as glucuronide conjugates, in the patient. In particular, total concentrations of daidzein and genistein in a sample collected shortly before dosing was 50 to 100 times greater than their average concentrations in a group of normal women sustained on a typical European omnivorous diet and approximately 10 times greater than found in Finnish women consuming a vegetarian diet [13].

Genistein has been shown to inhibit the growth of various cancer cell lines *in vitro* and *in vivo* without toxicity to normal cells. Genistein’s antitumor effect may be explained through interaction with the β estrogen receptor. Our institutional reagents only detect α ER, thus, it is difficult to know if the stabilization on this women’s tumor is due to interaction with the ER β receptor where its effects tend to be inhibitory [14]. The antitumor effects could be due to the inactivation of NF-kappaB activity [15] or alternatively mechanisms such as antiestrogenic effects of these phytoestrogens, inhibition of EGF-driven cell proliferation, inhibition of topoisomerase II, antiangiogenic effects, inhibition of PIP kinase, and increased TGF β 1 secretion [6]. In addition, in a prostate cancer cell line model, nanomolar concentrations of genistein inhibit TGF β 1-driven MMP2 expression and decreased cell invasion in

Boyden chambers perhaps through inhibition of the MAP kinase pathway [16]. Most recently, daidzein in combination with tamoxifen significantly delayed mammary tumor growth in Sprague–Dawley rats as compared to tamoxifen or tamoxifen with genistein [17]. Ovarian cancer cell lines exposed to genistein or daidzein at relevant concentrations show decreased proliferation [18,19]. Combination of genistein with quercetin or chemotherapy has demonstrated decreased colony formation and/or formation by independent investigators [9]. Mechanistic effects of this compound, however, are far from simple as demonstrated by recent preclinical studies with this compound that support its role in promoting or enhancing tumorigenesis in a variety of models including endometrial and vulvar neoplasm [20–24]. Clinical trials of genistein have predominantly focused on the phytoestrogenic effects on a variety of post-menopausal symptoms; however, treatment of men with red clover genistein prior to radical prostatectomy did demonstrate increased apoptosis in resected tumor specimens [19].

This case demonstrates the potential utility of genistein in platinum-resistant ovarian cancer. Further work with this complimentary therapy in ovarian cancer patients with limited treatment options is warranted, either potentially as a single agent, or as a complimentary agent in combination with standard chemotherapy.

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