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Contaminants of PC-SPES are not responsible for cytotoxicity in human small-cell lung carcinoma cells

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Abstract

PC-SPES is a mixture of herbs used in the treatment of prostate cancer. Batches of this product were found to contain traces of synthetic drugs, and the product was removed from the market. On the basis of a correlation between contaminant levels and cytotoxicity in prostate carcinoma cell lines, Sovak et al. [M. Sovak, A. Seligson, M. Konas, M. Hajduch, M. Dolezal, M. Machala, R. Nagourney, Herbal composition PC-SPES for management of prostate cancer: identification of active principles, J. Natl Cancer Inst. 94 (2002) 1275–1281] concluded that the contaminants were responsible for cytotoxicity of this preparation. Previously, we showed that extracts of PC-SPES are cytotoxic and pro-apoptotic in both drug-sensitive (H69) and drug resistant (H69V) human small-cell lung carcinoma (SCLC) cell lines. Here, we investigated whether the contaminants might be responsible for these effects. In contrast to the data reported for prostate carcinoma cells, extracts of batches of PC-SPES from the year 1998 (reportedly contaminated) and 2001 (much less contaminated) were equally cytotoxic in both SCLC cell lines. Tests of individual contaminants gave IC_{50} values far in excess of the amounts reported to be present in the IC_{50} level for the PC-SPES extracts: diethlystilbesterol: actual IC_{50} in H69 cells, >1000 μM; concentration present in herbal extract at IC_{50}, 0.05–0.2 μM; indomethacin: actual IC_{50} in H69 cells, 800 μM; concentration in herbal extract, 1.5–20 μM; warfarin: actual IC_{50} in H69 cells, 950 μM; concentration in herbal extract, 0.57–0.93 μM. Adding the calculated maximum concentration of the contaminants, singly or in combination, to extracts of the less contaminated batch (2001) of PC-SPES did not alter the cytotoxicity of the extract in H69 or H69V cells. At the contaminated concentrations, as well as 5× those concentrations, none of the contaminants was pro-apoptotic, as indicated by a DNA fragmentation kinetics assay. However, extracts of both early and late batches of PC-SPES were pro-apoptotic in SCLC cells. We conclude that the traces of pharmaceuticals found in PC-SPES were not responsible for its cytotoxic and pro-apoptotic activities of this herbal mixture on SCLC cells.

Keywords: Human lung cancer; Herbal extracts; Contamination; PC-SPES

1. Introduction

Small-cell lung cancer (SCLC) is particularly aggressive, with a 5-year survival rate at diagnosis rarely exceeding 15% [1,2]. SCLC treatment usually

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employs chemotherapy, with platinum-based combinations including etoposide, doxorubicin, 5-fluorouracil and taxol most common [3]. Unfortunately, drug resistance often occurs [4] and because there are numerous resistance mechanisms [5], this presents a major clinical challenge.

PC-SPES is a mixture of eight herbs that has been used successfully in the treatment of prostate cancer [6–8]. Extracts of this mixture are strongly cytotoxic and pro-apoptotic in prostate carcinoma cells [9]. Batches of PC-SPES were reported to have traces of synthetic drugs, including diethlystilbesterol (DES), indomethacin (IM) and warfarin (WF) [10–12]. As a result, the herbal mixture was removed from the market [13]. On the basis of a rough correlation between levels of contamination and cytotoxicity, Sovak et al. [10] concluded that the synthetic contaminants were responsible for the clinical effectiveness in prostate carcinoma.

We have shown that PC-SPES extracts are cytotoxic and pro-apoptotic in drug-sensitive and multi-drug resistant SCLC cells [14]. Here, we report investigations on the effects on SCLC cells of extracts of PC-SPES made from contaminated and less contaminated batches of the herbal mixture. Our data indicate that the contaminants were not responsible for the cytotoxic and pro-apoptotic effects.

2. Materials and methods

2.1. Cell lines

H69 human small-call lung carcinoma cells were grown as a suspension in AIM-V serum-free medium (Invitrogen-Gibco, Grand Island, NY) in 5% CO_2 atmosphere at 37 °C. A drug-resistant cell line (H69V) was selected in etoposide and grown in AIM-V. This cell line shows resistance to etoposide (9-fold), doxorubicin (10-fold) and vincristine (10-fold) [15].

2.2. Drugs, herbs and extracts

DES, IM and WF were obtained from Sigma Chemical Co., St Louis, MO and dissolved at appropriate concentrations in dimethyl sulfoxide. PC-SPES was obtained in 1998 and 2001 from the manufacturer, BotanicLabs, Brea, CA. The lots used in this study were 5438765 (manufactured about 06/1998) and 5430008 (manufactured about 05/2001). The contents of one capsule (320 mg) were dissolved in 1 ml of 95% ethanol for 1 h at 37 °C. After centrifugation at $3000 \times g$ for 10 min, the supernatant was filter sterilized (0.2 μm) and used immediately.

2.3. Cytotoxicity experiments

Extracts and/or drugs were added to logarithmically growing cells in 1 ml cultures containing 8000 cells/ml. After 4 days, cells were disaggregated mechanically and counted in a Coulter Z-1 counter. Counts were validated microscopically by hemocytometer after staining with trypan blue. All experiments were done in triplicate and repeated at least three times. IC_{50} was calculated as compared to solvent controls. Means were calculated and compared by two-tailed *t*-test.

2.4. DNA fragmentation test

The mechanism of cytotoxicity by herbal extracts and/or drugs was investigated by the kinetics of cellular DNA fragmentation (Roche Diagnostics kit, Indianapolis, IN). Briefly, 2×10^5 cells/ml were incubated for 16 h at 37 °C and 5% CO_2 in AIM-V medium with 10 μM BUdR to label DNA and then the cultures were incubated for 90 min in the herb/drug, after which 100 μl of the culture medium was removed for quantitation of released labeled DNA fragments by ELISA. This is a measure of cell necrosis. DNA fragments inside the cells (a measure of apoptosis) were measured following cell lysis in BSA–Tween 20 at 21 °C for 30 min. Following centrifugation, the lysate was used for ELISA.

For ELISA, a round-bottom microtiter plate was coated with anti-DNA antibody (mouse anti-human DNA monoclonal, clone MCA-33) overnight at 4 °C. Following blocking with BSA, 100 μl extract with the labeled DNA fragments was added to the coated wells and incubated for 90 min at room temperature. The DNA was fixed and denatured by microwave irradiation at 500 W for 5 min. Then