AN IN VITRO STUDY OF INHIBITORY ACTIVITY OF GOSSYPOL, A COTTONSEED EXTRACT, IN HUMAN CARCINOMA CELL LINES

MICHAEL L. LEBLANC a , JENNIFER RUSSO b , ANDRZEJ P. KUDELKA c and JUDITH A. SMITH c , d ∗

a University of Houston College of Pharmacy, Houston, TX, USA, b Department of Neuro-Oncology, UT M.D. Anderson Cancer Center, Houston, TX, USA, c Division of Cancer Medicine, Department of Gynecologic Medical Oncology, UT M.D. Anderson Cancer Center, Houston, TX, USA, d Division of Pharmacy, UT M.D. Anderson Cancer Center, Houston, TX, USA

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Gossypol, a cottonseed extract, has been shown to have antiproliferative activity in a variety of cancer cell lines. The objective of this study was to determine the inhibitory effects of gossypol on cell proliferation. Five human carcinoma cell lines were evaluated including endometrial (RL95-2), ovarian (SKOV-3), medullary thyroid (TT), and adrenocortical (NCI-H295R and SW-13). Gossypol and the metabolite, apogossypol hexaacetate, were examined at concentrations up to 500 µg mL⁻¹ and the IC₅₀ was determined using the MTT assay. Gossypol and apogossypol hexaacetate produced a dose-dependent growth inhibition in all cellular lines examined. The IC₅₀ for gossypol ranged from 1.3 to 18.9 µM while the IC₅₀ for apogossypol hexaacetate ranged from 5.2 to 9.0 µM. The results indicate that gossypol possesses antiproliferative action toward human carcinoma cells in vitro. These investigations suggest that gossypol may have therapeutic potential for the treatment of cancer.

INTRODUCTION

Gossypol, a polyphenolic aldehyde extracted in its racemic form from cottonseed, has been extensively investigated as a male contraceptive agent [1]. Recent evidence suggests that gossypol has potential as an anticancer drug because of its broad spectrum of inhibitory activity. Some of the inhibitory activities of gossypol include uncoupling of oxidative phosphorylation, stimulation and inhibition of respiration, and decreases in ATP production [2]. In addition, gossypol inhibits activity of enzymes in the electron transport chain, including lactic dehydrogenase X, NAD-isocitrate dehydrogenase, succinyl-CoA synthetase, as well as inhibiting other enzymes such as adenylate cyclase, ATPase, and phospholipid sensitive calcium-dependent protein kinase. Gossypol also affects the electrochemical properties of lipid membrane and the ordering of membrane lipid matrix by increasing their microviscosity and rigidity [3, 4].

The numerous inhibitory mechanisms of action of gossypol contribute to the antiproliferative activity of gossypol in both reproductive and nonreproductive cancer cell lines. The inhibitory actions of gossypol have been documented in vitro in human carcinoma cell lines including breast, ovarian, adrenocortical, and colon carcinoma cells. In vivo tests have demonstrated that gossypol treatment enhanced the survival of nude mice bearing Ehrlich ascites tumor and human adrenal cancer [5]. Furthermore, gossypol has been used in patients with metastatic adrenocortical cancer and demonstrated a 50% or greater decrease in tumor volume in 3 of 18 patients for a duration of 3 months to greater than a year [6].

After 72-h treatment with gossypol, cell growth was inhibited in several cancer cell lines of reproductive origin including the OVCA 420, 429, 432, and 433 ovarian cell lines, Tera-2 human embryonal carcinoma, and SK-UT-1 mesodermal tumor of the uterus. The IC₅₀ values of gossypol ranged from 0.86 to 1.98 µg mL⁻¹ [7]. A post-treatment decrease in DNA synthesis in these cells was determined by [3H]thymidine incorporation into DNA confirming inhibition of key enzymes such as DNA polymerase and topoisomerase II which are involved in DNA replication and repair [7]. These results have been reproducible in vitro in HeLa and Chinese hamsters ovary cells [8].

Corresponding author. UT M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 401, Houston, TX 77030, USA.
E-mail: jasmith@mail.mdanderson.org

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Gossypol has demonstrated inhibitory activity in a wide range of human carcinoma cell lines such as adrenocortical (SW-13), breast (T47D), prostate (Du-145), uterus (HeLa), and pancreas (Miapaca, RWP-2). However, further testing is required to elucidate inhibitory activity of gossypol and apogossypol hexacetate, an active metabolite, in resistant human carcinoma cell lines such as medullary thyroid carcinoma (TT) and other adrenal cortical carcinoma (ACC) steroid secreting (H295R) cell lines previously not investigated as well as confirm activity in the SW-13, a non-steroid secreting human carcinoma cell line. In 2002, there will be estimated 22,700 new cases of endocrine carcinomas reported associated with high mortality. For example, there is only estimated 2000 new ACC cases expected in 2002 resulting with approximately 1000 deaths [10]. Historically, endocrine tumors such as ACC and thyroid carcinomas have responded poorly to chemotherapy after failing primary surgical or radiation interventions leaving limited options for successful treatment of metastatic disease. Clinically, both medullary thyroid and ACC have shown poor response rates in patient populations treated with conventional therapeutic regimens, thus the need for new therapeutic options is important for improving patient survival. For instance, conventional chemotherapeutic agents such as cyclophosphamide, dacarbazine, and doxorubicin have response rates between 10 and 20% with the duration of response on average of only 1 year [6]. The toxicity associated with these agents include significant myelosuppression, nephrotoxicity, nausea and vomiting and some cardiotoxicity, especially with cumulative doses.

Gossypol was chosen for this study because preliminary preclinical and clinical data suggests less toxicity and potential efficacy demonstrated in other cancers. Furthermore, there is limited data reported on the activity metabolite, apogossypol hexacetate, in human carcinoma cell lines. The objective in this study was to evaluate the growth inhibitory activity of gossypol and also determine the potential growth inhibitory activity of the metabolite, apogossypol hexacetate, in five human carcinoma cell lines including (RL95-2) endometrial, (SKOV-3) ovarian, the potential growth inhibitory activity of gossypol and also determine metabolite, apogossypol hexaacetate, in resistant human carcinoma cell lines such as medullary thyroid carcinoma (TT) and other adrenal cortical carcinoma (ACC) steroid secreting (H295R) cell lines previously not investigated as well as confirm activity in the SW-13, a non-steroid secreting human carcinoma cell line.

**Methods**

**Supplies**

All human carcinoma cell lines were obtained from the American Type Culture Collection and maintained for less than 15 passages. Gossypol and apogossypol hexacetate, fetal bovine serum (FBS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO). The 75 cm² culture flasks and other cell culture supplies were obtained from Fisher Scientific (Pittsburg, PA).

Cell culture

Leibovitz’s L-15 medium supplemented with 10% FBS, 1% antibiotic, and 2 mM L-glutamine was used to maintain the small cell adrenal gland carcinoma cells (SW-13). The steroid producing adrenal gland carcinoma cells (NCI-H295R) were propagated in a 1:1 medium consisting of Dulbecco’s modified Eagle’s plus Ham’s F12. In addition, the medium was further supplemented with 15 mM HEPES, 6.25 μg ml⁻¹ insulin, 6.25 μg ml⁻¹ transferrin, 6.25 mg ml⁻¹ bovine serum albumin, and 5.35 μg ml⁻¹ linoleic acid, 97.5%; Nu-Serum I, 2.5%. Medullary thyroid carcinoma cells (TT) were maintained in Ham’s F12 medium supplemented with 1% antimycotic and 2 mM L-glutamine adjusted to contain 1.5 g l⁻¹ sodium bicarbonate and 10% FBS. Ovarian adenocarcinoma cells (SKOV-3) were maintained in McCoy’s 5a medium supplemented with 1.5 mM L-glutamine and 10% FBS. Endometrial carcinoma cells (RL95-2) were grown in a 1:1 mixture of Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F12 medium with 10 mM HEPES, 0.005 mg ml⁻¹ insulin and 2.0 g l⁻¹ sodium bicarbonate, 90% of 10% FBS, 1% antimycotic, and 2 mM L-glutamine. All cell lines were grown in 75 cm² culture flasks in 5% CO₂ in air at 37 °C to 90% confluence.

**Standard solutions**

Gossypol and apogossypol hexacetate, a primary metabolite, were dissolved in 1 ml DMSO to produce a final concentration of 40 mg ml⁻¹. All dilutions were completed with the respective cell culture media for each cell line in order to achieve concentrations from 0.01 to 500 μg ml⁻¹. The MTT stock solution was prepared by dissolving 54 mg of MTT in 20 ml PBS to achieve a final concentration of 0.3 mg ml⁻¹. Fresh standards and dilutions were made for each experiment.

**MTT assay**

Exponentially growing cells were detached from the cell culture by incubating with 1 x trypsin/EDTA for 5 min at 37 °C and a single cell suspension was produced. An appropriate dilution of the cells was made to achieve 7000 cells per well for SKOV-3, SW-13, and RL95-2. To achieve confluence, an increased number of cells, 20,000 and 25,000, were required to conduct studies in the TT and H295R cell lines, respectively. Plated cells were incubated for a 24 h period prior to treatment. Cells were treated with concentrations ranging from 0.01 to 500 μg ml⁻¹ of gossypol and apogossypol hexacetate. Each concentration of gossypol and metabolite were completed in quadruplicate. Also, duplicate cell blank (no drug) and drug blank (no cells) controls were included for each 96-well plate. After 72 h treatment, MTT was added to each well of the five plates, and then incubated for 2 h at 37 °C. The plates were centrifuged at 1.5 K for 5 min then the media was removed. To stop the reaction 50 μl of DMSO was added and absorbency measured at 570 nm within the hour. The experiments were repeated.
in triplicate and the IC₅₀ was determined from the mean percent growth inhibition.

RESULTS

Both gossypol and apogossypol hexaacetate induced a dose-dependent decrease in cell viability in all cell lines studied. Gossypol and apogossypol hexaacetate had activity in all cell lines studied. The cytotoxicity after 72h exposure to gossypol and apogossypol hexaacetate is illustrated by the growth inhibition curves in [Fig. 1(A–E)] for the five cell lines. The IC₅₀ for gossypol ranged from 1.3 to 18.9 µM in the five cell lines. Apogossypol hexaacetate had similar growth inhibition activity with the IC₅₀ for apogossypol hexaacetate ranging from 5.2 to 9.0 µM.

DISCUSSION

Previous reports in the literature have demonstrated that gossypol possesses in vitro antiproliferative activity in a variety of carcinoma cell lines and our results support these
findings. Gossypol produced a dose-dependent decrease in cell viability in all of the carcinoma cell lines studied and this cytotoxic activity is similar to activity seen in other cell lines previously evaluated [9]. The results from this study demonstrate that human carcinoma cell lines such as RL95-2, SKOV-3, NCI-H295R and SW-13, and TT are sensitive to the antiproliferative effects of gossypol.

The metabolite, apogossypol hexaacetate, had similar growth inhibitory effects to gossypol in all carcinoma cellular lines evaluated. This data demonstrates apogossypol hexaacetate is an active metabolite of gossypol and contributes to the antiproliferative effects. Additional studies are needed to determine the significance of this data.

This investigation of gossypol and apogossypol hexaacetate in human carcinoma cell lines indicates that gossypol and apogossypol hexaacetate have antiproliferative activity and suggest that they may have therapeutic potential for a variety of carcinomas. Additional experiments are ongoing to further delineate the mechanism of gossypols cytotoxic effects. In conclusion, this initial in vitro data confirms the potential inhibitory effects of gossypol, and its metabolite, and supports the need to pursue clinical studies to evaluate it as a potential new anticancer agent.

![Graphs showing the growth inhibition of gossypol and apogossypol hexaacetate at different concentrations.](C)
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REFERENCES
