Aloe Emadin’s Effects on Apoptosis in Cervical Cancer Cells

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ABSTRACT
Aloe emadin, an anthraquinone found in the gel of Aloe vera leaves, has been shown to have antineoplastic properties in cancer lines without generalized cytotoxic effects on healthy tissue. The apoptotic effects of aloe emadin were studied in comparison to cisplatin, a commonly used chemotherapeutic drug. The apoptotic characteristics of the compounds were evaluated by regression analysis of cell viability on cervical cancer cells (HeLa) and observation of changes in morphology, prostaglandin E2 (PGE2) levels via the COX-2 pathway, and DNA ladder formation. Observation of the changes after 24, 48, and 72 hours of the administration of aloe emadin at LD25 and LD50 concentrations strongly indicate the induction of apoptosis in HeLa cancer cell line. Treatment with aloe emadin resulted in significant changes in morphology typical of apoptosis, and the formation of DNA ladders visualized by gel electrophoresis. Preliminary data also suggested reduction in PGE2 levels via mass spectrometry analysis following aloe emadin treatment of HeLa cells. Regression analysis of cell viability showed aloe emadin to reduce viability by 37.6% compared to cisplatin in the first 24 hours at LD50, and by 71.3% at 48 hours. Our results indicate that aloe emadin is a very promising novel chemotherapeutic drug that may improve the future treatment of cancer by increasing effectiveness while reducing the negative side effects.

Key Words: Cancer, Aloe vera, Aloe emadin, apoptosis, chemotherapy

INTRODUCTION
Cancer cells differ from healthy tissue cells by undergoing the process of dedifferentiation and dividing uncontrollably into cell masses that form tumors and may metastasize to other parts of the body. The transformation of a normal cell to a cancerous one is typically caused by metabolic abnormalities, environmental factors, or genetic predispositions.1 Healthy cells have checkpoints throughout their life cycles to ensure that the feedback controls that regulate cell proliferation are functioning properly.2 When abnormality is detected, cells will undergo apoptosis to eradicate themselves as a threat.3 Apoptosis signaling regulates cells in the body, but cancer cells have lost the ability to recognize these signals.4,5 Cancerous cells can hide from these checks through activation of the COX-2 pathway and production of prostaglandin E2 (PGE2), which inhibits apoptosis and promotes proliferation, invasion, and assists with metastasis.6 Chemotherapies used to treat cancer are often antiproliferative, antitumor drugs. These drugs, including cisplatin, a platinum-based antitumor drug,7 exert their antiproliferative effects through the induction of apoptosis. Unfortunately, many of the chemotherapies used today do not specifically target cancer cells alone, but produce unintended cytotoxic effects to the healthy tissue adjacent to them.8,9 Furthermore, some cancer cells, such as cervical cancer cells, become resistant to their effects and may mutate their genes to oppose the intended effects of the drugs.7,10 Aloe vera has been used in traditional medicine for centuries for the treatment of a variety of ailments such as herpes, asthma, and diabetes.11 There is some evidence that crude extracts from Aloe vera can act synergistically with chemotherapeutic drugs reducing cytotoxicity.12 Aloe emadin, an anthraquinone compound present in the leaves of Aloe vera, has exhibited anticancer biological function in esophageal, colon, pancreatic, and many other types of cancers.13 Aloe emadin seems to target cancerous cells by down regulating many key cancer promoting molecules without cytotoxic effects,13-16 but no studies have yet explored its effects on PGE2 regulation. It was the purpose of this work to explore the effect of Aloe vera components as alternative to commonly used chemotherapies, as potential inducers of apoptosis and inhibitors of the COX-2 pathway in cervical cancer, HeLa cells. Therefore, Aloe emadin has the potential to become a suitable alternative to the drugs used to treat cervical cancer.

METHODS
Cell Culture
Cervical cancer derived HeLa cells (CCL-2™, ATCC®, Manassas, VA) were cultured in multi-well plates, in Dulbecco’s Modified Eagle Media (DMEM, Corning), supplemented (DMEM+) with 1% antibiotics (Penicillin, Streptomycin, and Amphotericin, Corning), 10% Fetal Bovine Serum (FBS, Gibco), and 2mM L-Glutamine (Corning). Cells were seeded at a concentration of 1.0x106 cells/mL and grown for 24 hours at 37°C, 5% CO2, and 95% humidity.

Aloe Emodin, Aloe vera Gel, Celecoxib, and Cisplatin Stock Solutions and Administration
Aloe emadin (Torcris Bioscience) and Aloe vera (FGCU Food Forest) were the extracts tested. Cisplatin (Torris Bioscience) served as a control and Celecoxib (TSZ Chem) as a pathway enhancer. Aloe emadin and celecoxib were dissolved in 1% dimethylsulfoxide (DMSO, SIGMA) while cisplatin was dissolved in sterile deionized water to create 1mM stock solutions.13 Aloe vera was collected from the FGCU Food forest and its freshly harvest-
ed leaves washed under running tap water for 10 minutes. Aloe gel was scraped from the leaves and diluted with sterile deionized water in a 1:4 ratio. The stock solutions of aloe emodin, celecoxib, and cisplatin were further diluted in 1:10 ratios to use workable and accurate volumes for the concentrations determined. Following the protocols of Chen et al., a range of concentrations for aloe emodin, celecoxib and cisplatin were established as follows: 2.5, 5.0, 10.0, 20.0, 40.0 μM for emodin, 15.0, 30.0, 40.0, 50.0, 70.0 μM for cisplatin, and 5.0, 1.0, 20.0, 30.0, 40.0 μM for celecoxib. It should be noted that due to equipment limitations, we were not able to successfully purify the Aloe vera gel into workable volumes. The effects of the various concentrations of aloe emodin, celecoxib, and cisplatin were tested on HeLa cells growing in 6 well tissue culture plates (Falcon®). Cells were treated with the corresponding chemicals, one concentration per well; one well was left untreated to serve as a control. The cells were checked at 24, 48, and 72 hours after treatment to see the effects of aloe emodin, cisplatin, and celecoxib on cell division. The experiments were repeated three times.

Cell Viability
The supernatant was removed from the cell cultures; cells were washed with 1mL of phosphate-buffered saline (PBS, Corning) to remove the left over DMEM+ followed by the addition of 1mL of trypsin (0.05% 0.53 mM EDTA, Corning) per well to detach the cells from the plates. Once the cells were completely detached, 500μL of unsupplemented DMEM was added to the cells to counteract any effects trypsin may have on the cells. 50μL of the cells were collected and combined with 50μL of trypan blue dye (0.4% w/v, SIGMA) to stain the dead cells. 10μL of this solution was loaded onto a hemocytometer and counted to compare the living cells against the dead cells. The equation below was used to determine the cell viability:

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\text{(No. Viable Cells Counted)/(No. Total Cells Counted)} \times 100\% = \text{% Viable Cells}
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Results of the cell viability assays for the different chemicals and their respective concentrations were calculated and the general relationship of aloe emodin, celecoxib, and cisplatin concerning their effectiveness towards cell viability were composed (Figures 1, 2, and 3). This allowed us to determine the lethal dose 25 and 50, which are the concentrations that resulted in a 25% and 50% reduction in cell viability according to the protocol described by Chou et al. Analysis of DNA Fragmentation (DNA Ladder) through Gel Electrophoresis
After 24 and 48 hours of treatment, cells were washed with ice cold PBS and resuspended in Wizard® SV Lysis Buffer. DNA was extracted using the Wizard SV genomic DNA purification system (Promega, Madison, WI), according to the manufacturer’s instructions. Electrophoresis was performed on 1% agarose gel with a 1kB DNA marker (Promega) in combination with the nucleic acid stain Gel-RedTM, which aided in the visualization of DNA (1 μg/lane) under UV light.

Figure 1: Aloe emodin’s effect on cell viability at varying concentrations was used to determine the LD25 (6.7μM) and LD50 (19.9μM) concentrations.

Figure 2: Celecoxib’s effect on cell viability at varying concentrations was used to determine the LD25 (10.3μM) and LD50 (22.9μM) concentrations.

Figure 3: Cisplatin’s effect on cell viability at varying concentrations was used to determine the LD25 (16.9μM) and LD50 (38.7μM) concentrations.

Prostaglandin E2 Content Determination
After 24 hours of treatment, 2mL of growth media was collected and precipitated with 2mL of ethyl acetate, followed by centrifugation at 1,000 rpm for 1 minute. The organic layer was collected and transferred to a new test tube. The samples were vacuum dried, resuspended in methanol, and their prostaglandin E2 content determined through liquid chromatography mass spectrometry with electrospray ionization on TSQ triple quadrupole instrument with a parent mass of 350.68 m/z and product mass of 271.5 m/z.
RESULTS

The concentrations, lethal doses (LD), which resulted in a 25% reduction (LD25) and 50% reduction in cell viability (LD50) were calculated for aloe emodin, celecoxib, and cisplatin. The corresponding LD25 concentrations were 6.7 μM, 10.3 μM, and 16.9 μM; while the LD50 were 19.9 μM, 22.9 μM, and 38.7 μM (Figures 1, 2, and 3). Morphological changes on HeLa cells in response to these treatments were observed under a phase contrast microscope. The cells shrank considerably and showed blebbing (formation of apoptotic bodies) around the plasma membrane (Figure 4). Cell viability was reduced by 37.6% and 71.3% compared to the control after 24 and 48 hours of exposure to LD50 aloe emodin respectively (Figure 5). In general, aloe emodin was more effective than the control (cisplatin) at lower concentrations; when comparing 40 μM of aloe emodin to 70 μM of cisplatin, aloe emodin was 14.8% better at reducing viability. DNA purified from the treated cells showed fragmentation when run on a 1% agarose gel (Figure 6). It should be noted that with Aloe vera fresh extract we were not able to successfully purify the gel into workable volumes, and that the results concerning PGE2 inhibition were inconclusive.

DISCUSSION

Through our preliminary findings we were able to describe some of the effects that aloe emodin has on cervical cancer cells. Indication of apoptosis is strongly supported due to characteristic morphological changes in the cell and creation of the DNA ladder after 48 hours of exposure with aloe emodin (Figures 4 and 6). At LD50 concentrations, aloe emodin shows a dose-and-time dependent induction of apoptosis, as seen similarly in Chen et al.17 and Pecere et al.16 findings. Aloe emodin is comparable to cisplatin in inducing apoptosis, but more effective in reducing cell viability (37.6% and 71.3%) at both 24 and 48 hours after treatment. However, more experiments are needed to evaluate the cytotoxicity of aloe emodin on healthy cells and selectivity on cancer cells.
REFERENCES