Original Research Article

Effect of *Schisandra chinensis* (Turcz) Schisandraceae seed extracts and cisplatin on cytotoxicity, genotoxicity and wound healing in MCF-7 cells

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Abstract

Purpose: Schisandra chinensis is a plant used in traditional Chinese and Russian medicine. An S. chinensis seed extract was tested for its ability to potentiate the effects of the anticancer agent cisplatin in MCF-7 breast cancer cells.

Methods: S. chinensis seeds were extracted with ethanol and the ethanol was evaporated from the extracts to obtain an aqueous fraction of the S. chinensis seed extract (SCSE). MCF-7 cells were exposed to cisplatin alone or in combination with various concentrations of SCSE. The end points that were measured were cytotoxicity, genotoxicity, and wound healing.

Results: The addition of 10 % SCSE increased the cytotoxicity of cisplatin by increasing MCF-7 cell death by 7 %. The combination of 20 % SCSE and cisplatin completely inhibited wound healing in MCF-7 cells. SCSE alone did not induce DNA fragmentation in MCF-7 cells.

Conclusion: Compounds from S. chinensis seed extracts may mitigate cancer cell proliferation and migration.

Keywords: Schisandra chinensis, MCF-7 cells, Cytotoxicity, Genotoxicity, Wound healing, Cisplatin

INTRODUCTION

Schisandra chinensis is a dioecious plant that is native to northern China and several regions of Russia. *S. chinensis* is cultivated for its berries, which are used in teas, wines, confectionaries, and as flavoring agents. The berries are known as 'five-flavored berries' for their combination of five tastes. *S. chinensis* is considered an adaptogen because it is beneficial to human health, does not cause side effects or toxicity, and is one of the 50 herbs that are fundamental to traditional Chinese medicine [1]. The seeds of *S. chinensis* are used to treat respiratory, urinary, hepatic, nervous, and digestive ailments [2]. *S. chinensis* is also used for medicinal purposes in Russia, where scientific studies of *S. chinensis* are well documented [3]. Extracts of *S. chinensis* contain high levels of schisandrin B, which has significant antichlamydial activity and is safe for humans [4].

The phytochemical composition of *S. chinensis* and the physical and chemical properties of its primary compounds are well known [5–7]. The primary beneficial compounds in *S. chinensis* are the lignins schisandrin and gomisin A, which play important roles in the adaptive immune response [8]. Lignins stimulate the production of cytokines, including interleukin-8, granulocyte-macrophage colony-stimulating factor, and macrophage inflammatory protein-1β [9].

S. chinensis seed extracts (SCSEs) have been shown to inhibit the growth of cultured human breast cancer cells by arresting the cell cycle [10]. The *in vivo* effects of SCSEs have also been studied using mouse and rat models. Multiple studies have demonstrated the hepatoprotective effects of SCSEs [11–13] and have evaluated the toxicity of gomisin A in rats [14–16].

MCF-7 is a human breast cancer cell line that is routinely used to evaluate drug actions, tumor microenvironments, and drug efficacy [17]. MCF-7 has been used to study the effects of cisplatin in combination with a modulatory agent, ginsenoside compound K [18]. In this study, we tested the combinatorial effects of SCSEs and cisplatin on MCF-7 cells, and we used genotoxicity and wound healing as the evaluation endpoints.

EXPERIMENTAL

Plant materials and extracts

An SCSE was obtained by soaking 500 g of air-dried *S. chinensis* seeds with 95 % ethanol ten times as described previously [19]. The SCSE was filtered and the ethanol was evaporated. After evaporation, a 50 mL aqueous fraction remained. The aqueous fraction was filter-sterilized using a 0.22 µm filter, aliquoted, and stored at 4 °C until further use.

Cell culture

The MCF-7 cell line was cultured as a monolayer in DMEM supplemented with 10 % fetal bovine serum. Cells were seeded in 1 mL of medium in 24-well plates at a density of 0.12×10^6 cells/mL. The cytotoxicity, genotoxicity, and wound healing experiments were performed in duplicate.

Evaluation of cytotoxicity and genotoxicity

Cisplatin-induced cytotoxicity and genotoxicity were evaluated by treating cells with 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, or 30 µM cisplatin for 24 h. The trypan blue assay was used to evaluate cytotoxicity and DNA fragmentation. After exposure to cisplatin, cells were trypsinized and harvested, and the trypan blue assay with the Neubauer chamber was used to calculate the percentages of live and dead cells. The half-maximal inhibitory concentration (IC_{50}) value for cisplatin-induced cytotoxicity were determined. In addition, DNA was extracted from cells that were exposed to various concentrations of cisplatin using the standard salting-out method. DNA concentration and purity were measured using a NanoDrop™ spectrophotometer. DNA electrophoresis was performed using standard agarose gels and gels were viewed using a Gel Doc™ image analysis system.

Cells were cultured in 24-well plates and incubated with 25, 27.5, or 30 µM cisplatin for 24 h. After incubation, the wells were washed three times with 1 mL of phosphate-buffered saline. Then, the cells were then scratched with a 200 µL pipette and wound healing was monitored 24 h later.

Cells were cultured in 24-well plates and were incubated with 2, 5, 10, 15, or 20 % SCSE (v/v) for 24 h. After incubation, cells were harvested for cytotoxicity and genotoxicity assays. Cells were cultured in 24-well plates and were incubated with 11.3 µM cisplatin and 2, 5, 10, 15, or 20 % SCSE (v/v) for 24 h. Following incubation, the cells were harvested for cytotoxicity and genotoxicity assays. Wound healing assays were performed with cells that were incubated with a combination of 11.3 µM cisplatin and 2, 5, 10, 15, or 20% SCSE (v/v) for 24 h. Following incubation, the wounds were analyzed to determine healing rates.

Statistical analysis

All of the experiments were performed in triplicate and values were averaged. IC_{50} values were plotted as graphs with standard deviations, error bars, and R-values. DNA fragmentation and wound healing data are presented as images.

RESULTS

Cisplatin-induced cytotoxicity, genotoxicity, and wound healing

Trypan blue assays revealed that the IC_{50} of cisplatin in MCF-7 cells was 11.3 µM (Figure 1). Cisplatin concentrations of 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, or 25 µM did not induce DNA fragmentation, whereas 27.5 and 30 µM cisplatin induced DNA fragmentation in MCF-7 cells (Figure 2).
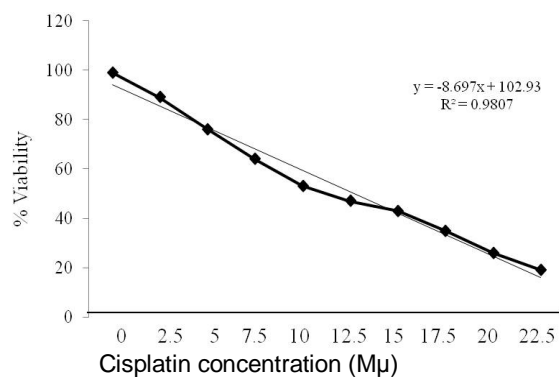
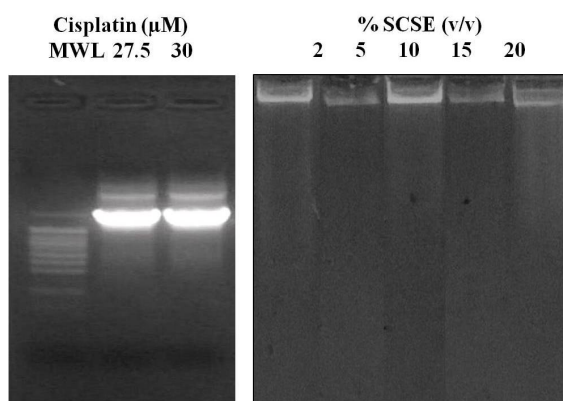**Figure 1:** Effect of cisplatin on cell viability

Figure 2: Effect of cisplatin SCSE on DNA fragmentation in MCF-7 cells. MCF-7 cells were incubated with 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, or 30 µM cisplatin, and 27.5 and 30 µM cisplatin induced DNA fragmentation, whereas lower cisplatin concentrations did not induce DNA fragmentation. MCF-7 cells were incubated with 2, 5, 10, 15, or 20% SCSE, and none of these SCSE concentrations induced DNA fragmentation indicating that SCSE is not toxic to MCF-7 cells. MWL indicates the molecular weight ladder

The wounds on control cells healed fully within 24 h, whereas the wounds on cells treated with 25, 27.5, or 30 µM cisplatin did not heal completely (Figure 3).

Effect of SCSE on MCF-7 cells

Cells exposed to 2, 5, 10, 15, or 20 % SCSE for 24 h exhibited wild-type morphologies, culture characteristics, viability, DNA integrity, and wound healing.

Effect of cisplatin and SCSE on MCF-7 cells

The IC$_{50}$ of cisplatin on MCF-7 cells was 11.3 µM, however cytotoxicity increased when cells were treated with 11.3 µM cisplatin and 2, 5, 10, 15, or 20 % SCSE (v/v) for 24 h. Treatment with 11.3 µM cisplatin and 10 % SCSE (v/v) resulted in 43 % MCF-7 cell viability (Figure 4), however, cell viability did not decrease further when 11.3 µM cisplatin was combined with 15 or 20 % SCSE.

DNA fragmentation was not observed in cells treated with 0, 2, 5, 10, 15, or 20% SCSE (v/v) and 11.3 µM cisplatin. Incomplete wound healing was observed in cells that were cultured with 11.3 µM cisplatin and 2, 5, or 10% SCSE (v/v) for 24 h. No wound healing was observed in cells that were treated with 11.3 µM cisplatin and 15 or 20 % SCSE (v/v) for 24 h.

DISCUSSION

S. chinensis is well known for its medicinal properties and has been used in traditional medicine, especially in China and Russia.
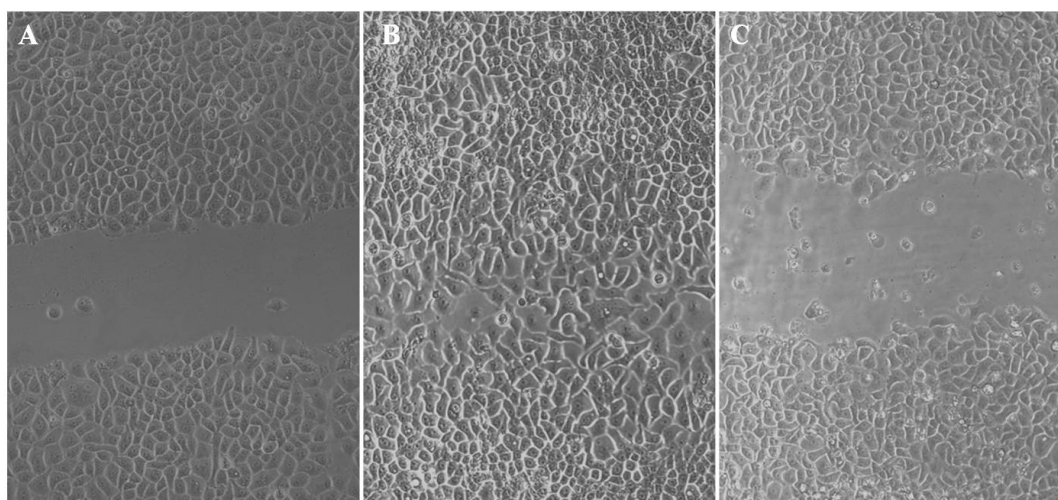

Figure 3: Effect of cisplatin SCSE on wound healing in MCF-7 cells. (A) Control MCF-7 cells with an induced wound at time 0. (B) Control MCF-7 cells showed complete wound healing after 24 h. (C) MCF-7 cells treated with 20% SCSE (v/v) and 1.3 µM cisplatin showed incomplete wound healing after 24 h
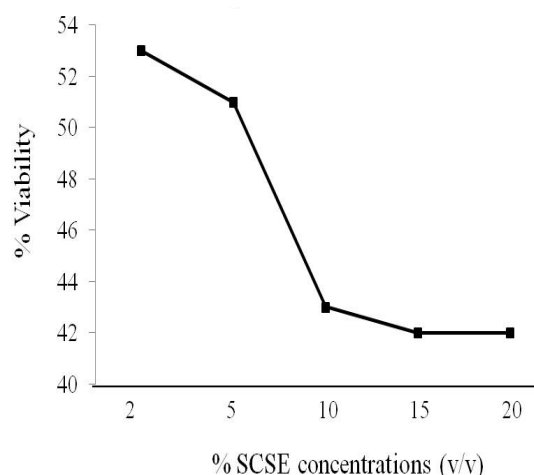
Figure 4: Effect of a combination of cisplatin and SCSE on cell viability. IC_{50} of cisplatin on MCF-7 cells was 11.3 µM. MCF-7 cell cytotoxicity increased upon the addition of SCSE with 11.3 µM cisplatin. A combination of 15 or 20 % SCSE and 11.3 µM of cisplatin reduced MCF-7 cell viability to approximately 43 % compared to 53 % for cisplatin alone

The active compounds in *S. chinensis* seeds have been characterized and several animal studies have shown the beneficial effects of *S. chinensis* phytochemicals, for example, it has been shown that *S. chinensis* extracts can induce cell-cycle arrest in breast cancer cells [10]. *S. chinensis* extracts have also been tested in combination with other chemotherapeutic agents, including carbon tetrachloride, halothane, 3'-methyl-4-dimethylamino-azobenzene, deoxycholic acid, hepatotoxins, hydrogen peroxide, and doxorubicin [20]. In this study, we showed that SCSE modulates cisplatin-induced cytotoxicity in MCF-7 cells, thus supplementation with SCSE could reduce the cisplatin concentration required to reach the IC_{50} value. We initially hypothesized that SCSE may retard wound healing in MCF-7 cells indicating a potential role in the control of cancer cell migration and metastasis, however, we did not observe any genotoxic effects of SCSE on MCF-7 cells. Rather, we found that SCSE may be useful in cancer therapeutics because it is non-toxic and augments the cytotoxicity of the chemotherapeutic drug cisplatin.

CONCLUSION

Cisplatin is a commonly used cancer chemotherapeutic drug, which induces cellular apoptosis through the formation of DNA adducts, and the MCF-7 cell line is also widely used in cancer research, thus we chose cisplatin and the MCF-7 cell line for this study. In this study, we demonstrated the potential of SCSE as a useful adjuvant for chemotherapy that could reduce the doses of chemotherapeutic drugs given to patients. Nevertheless, *in vivo* studies are still required to determine the translational potential of SCSE to human clinical applications.

DECLARATIONS

Acknowledgement

Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Both authors contributed equally to the experimental design, data collection, interpretation of results, and manuscript preparation.

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