Brief Report

Schizopeltic Acid Inhibits the Growth of Murine Polyploid Pulmonary Blastoma Cells *in vitro*

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***Abstract*:** We tested the effects of Schizopeltic acid, a secondary metabolite of the lichen *Collema quadriloculare*, on the growth of murine polyploid pulmonary blastoma cells in vitro. We found that Schizopeltic acid was a potent inhibitor of growth. We also found that Schizopeltic acid increased sensitivity of cells to radiation, and this effect was significant at radiation intensity lower than the standard intensity of cancer radiotherapy. Results of this study indicate that Schizopeltic acid shows promise for combined-modality cancer treatment.

Keywords: cancer, irradiation, schizopeltic acid, polyploidy pulmonary blastoma cells

1. Introduction

Reinfection of tissues with cancer cells that have acquired radioresistance during treatment is a great challenge for cancer radiotherapy [1]. As such, radiotherapy is often applied in combination with chemotherapy. The most effective of chemotherapeutic drug combinations should inhibit growth of the cancer cell and also increase sensitivity of cancer cells to radiation. Moreover, the radiosensitizing effect should also enhance radiotherapy at low radiation intensity. Therefore, radiotherapy in combination with chemotherapy (combined-modality treatment) is the best standard of treatment for most cancers [2]. As the discovery of effective anti-cancer drugs is a very slow process [3], the secondary metabolites of the lichens as possible drugs for cancer therapy have been intensively explored. This study was thus designed to investigate the biological activity of Schizopeltic acid, a secondary metabolite of the lichen *Collema quadriloculare*.

Lichens are a symbiotic assemblage of plant and fungus. Because of their social arrangement, and because of the diversity and the complexity of their ecological niches, lichens produce so many chemicals for unique colors, signaling between symbionts, manipulation of UV light, and defense against the foragers. Although more than 700 secondary metabolites of lichens have been isolated, only a small number of them have been characterized for biological activity [4].

Cancer is a complex disease that begins with the uncontrolled growth of the cells. A cancer cell does harm by forming tumors, absorbing tissues, and spreading through the body by metastasis. The highest probability of survival from cancer is with strong inhibition of proliferation of the cancer cells at the beginning of this progression [5]. Therefore, the establishment of the inhibition of proliferation of cancer cells *in vitro* is a critical first step for drug discovery. In our attempt to determine the biological activity of Schizopeltic acid, we tested its effect on the growth of murine polyploid pulmonary blastoma cells *in vitro*. In addition, we also tested its effect in combination with irradiation with a range of intensities.

2. Materials and methods

2.1. Chemicals

The chemical structure of Schizopeltic acid is shown in Figure 1. Pure extracts were dissolved and serially diluted in a 2:1 mixture of ethanol and phosphate buffered saline (EtOH / PBS, pH 7.4). These solutions were added as aliquots of 0.01 ml to 0.99 ml to cell cultures to achieve the final concentrations of Schizopeltic acid: 10 µM, 1 uM, 0.1 µM, 0.01 µM, 0.001 µM, and 0.0001 βM. The control group received 0.01 ml of growth medium.

2.2. Cells and cell culture

Murine polyploid pulmonary blastoma cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mg/ml N-2-hydroxyethylpiperazine -N'-2-ethanesulfonic acid, 100 U/ml penicillin G, 0.1 mg/ml streptomycin, 2 mg/ml sodium bicarbonate, and 5% fetal bovine serum (FBS). Cell cultures were washed with PBS, then

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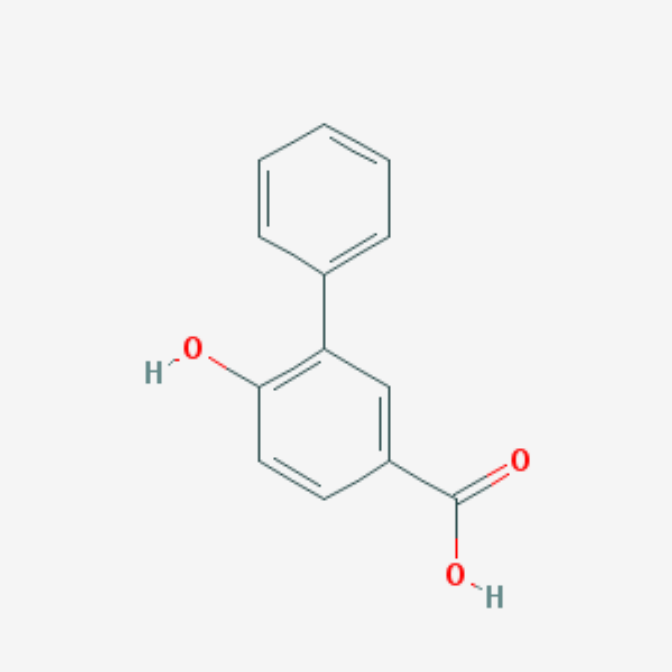

Fig. 1. The chemical structure of Schizopeltic acid.

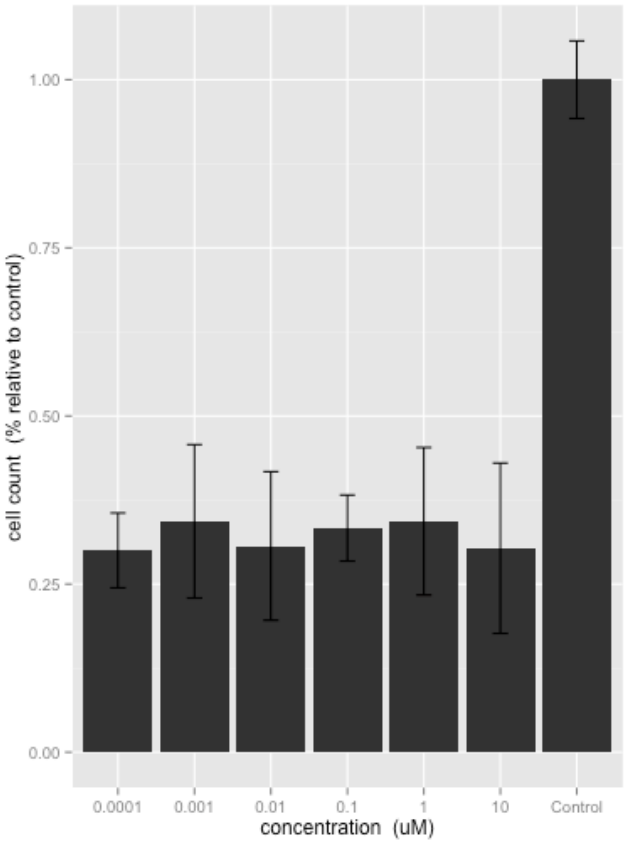

Fig. 2. Threshold effect of Schizopeltic acid on the growth of murine polyploid pulmonary blastoma cells. The X axis shows concentration (μM) Schizopeltic acid in culture tubes before growth. The Y axis shows cell count after 5 days of growth, normalized to that of the control. Confidence intervals at 95% are indicated. The difference between 0.0001 μM Schizopeltic acid treatment and control is significant ($p < 0.001$).

treated with 0.2% trypsin/PBS, and then washed with RPMI 1640 medium and centrifuged. The cell pellet was resuspended in RPMI 1640 medium and washed with more medium and the cells were counted. Schizopeltic acid solutions were aliquoted to cells in 24-well plates. The treated cells were then cultured in 100-mm plastic tissue-culture dishes at 37 ^{o}C with 5% CO_2 under high humidity. The final cell counts were measured after 5 day's growth.

2.3. Irradiation

Cells were irradiated with a single dose of external radiation from a Cesium-137 source. Doses in the range of 0.5 to 15 Gy were used. The dose rate was 1 Gy per 4 seconds. A control group received no radiation.

2.4. Data analysis

Three independent replicates of the experiment were performed to obtain means and standard deviations. Mean cell counts were normalized to that of control cells grown in parallel. Significance of differences between treatments was determined by analysis of variance and Student's t-tests using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria). A p-value of <0.01 was accepted as significant.

3. Results

3.1. Threshold effect of Schizopeltic acid on the growth of the rat glioblastoma cell

We cultured the cells in parallel with doses of Schizopeltic acid at different concentrations. We measured the cell proliferation after 5 days in the logarithmic growth phase. Figure 2 shows the results of the first experiment. All concentrations of Schizopeltic acid had a similar level of effect. All concentrations caused a significant inhibition of cell growth when compared with that of the control. Cell growth was inhibited with treatment at the lowest concentration of Schizopeltic acid (0.0001 μM), which caused 70% slower proliferation than that of the control ($p < 0.001$).

3.2. *Effect of Schizopeltic acid in combination with irradiation on the growth of murine polyploid pulmonary blastoma cells*

With the results of the first experiment, we further tested the lowest concentration of Schizopeltic acid (0.0001 μM) in combination with gamma radiation. We grew the cells under the same condition as described in Fig. 2, but with the following modification. Again, pure extracts were dissolved and serially diluted in a 2:1 mixture of ethanol and phosphate buffered saline (EtOH / PBS, pH 7.4). These solutions were

added as aliquots of 0.01 ml to 0.99 ml to cell culture to achieve the final concentration of Schizopeltic acid (0.0001 µM). The control group received 0.01 ml growth medium in the absence of irradiation.

Figure 3 shows the results of the second experiment. Lower than nanomolar concentration of the Schizopeltic acid powerfully enhanced the inhibitory effect of radiation on cell growth. This effect was significant at 0.5 Gy, the lowest level of radiation ($p = 0.0012$).

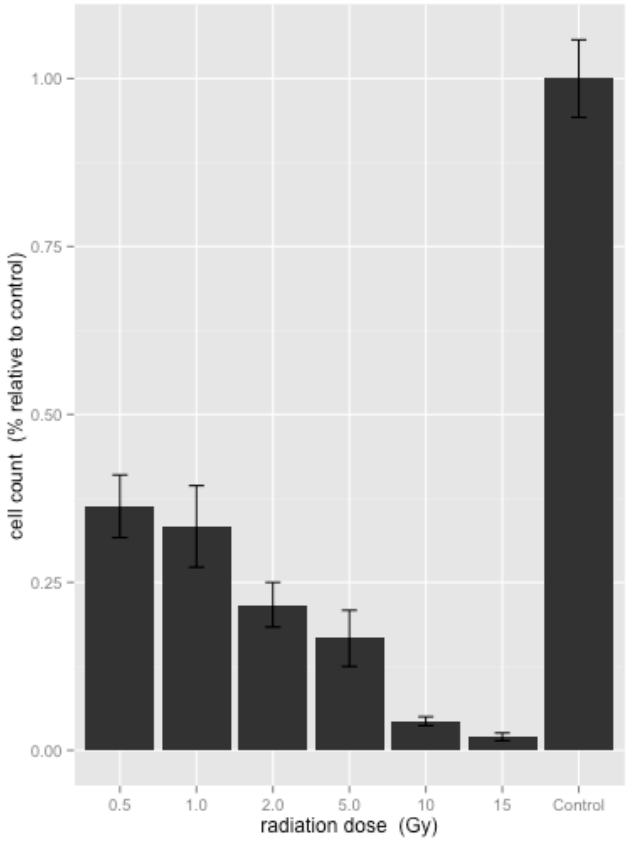

Fig. 3. Effect of Schizopeltic acid in combination with irradiation on the growth of murine polyploid pulmonary blastoma cells. The X axis shows intensity (Gy) of radiation. The Y axis shows cell count after 5 days of growth, normalized to that of the control. Cells were irradiated after treatment with 0.0001 µM Schizopeltic acid. Confidence intervals at 95% are indicated. The difference between 0.5 Gy and control is significant ($p = 0.0012$).

4. Discussion

In this study, we have tested the biological activity of Schizopeltic acid, a secondary metabolite of the lichen *Collema quadriloculare.* Specifically we measured the effect of this compound on growth of murine polyploid pulmonary blastoma cells in vitro.

Our results show that Schizopeltic acid inhibits cell growth. The mechanism of action is unknown, but the effect is potent. Even at the lowest dose (0.0001 uM), Schizopeltic acid has a significant negative effect on cell growth in vitro after 5 days of logarithmic growth when compared to the control cells.

To determine if the inhibitory effect interacted with gamma radiation, we tested the rat glioblastoma cell with 0.0001 µM Schizopeltic acid and a range of radiation intensities. The result demonstrates that Schizopeltic acid is also a radiosensitizer. Therefore, Schizopeltic acid enhanced the inhibitory effect of radiation on the growth of cancer cells. This effect was significant at 0.5 Gy, a radiation dosage that is lower than the standard radiation dosage in cancer radiotherapy.

We propose that the biological activity of Schizopeltic acid is related to lichen ecology. It is known that lichens are adapted for the manipulation of radiation, and also adapted for defense against the foragers [6]. Therefore, it is not surprising that the secondary metabolites of the lichen can enhance the effect of radiation and inhibit foreign cells.

Our study is the first to demonstrate that Schizopeltic acid is a radiosensitizer with anti-cancer activity. Results of the present studies suggest that Schizopeltic acid is a promising new drug for the combined-modality treatment of cancer. In future studies, we will need to demonstrate that Schizopeltic acid is an effective agent against cancers in animals and humans.

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Conflict of interest: None declared.

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