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Oxyskyrin Inhibits the Growth of Murine SV40 Transformed Bone Blastoma Cells in Vitro

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Abstract

We test the effects of Oxyskyrin, secondary metabolite of the lichen *Lecanora pseudistera*, on the growth of murine SV40 transformed bone blastoma cells in vitro. We find that Oxyskyrin is a potent inhibitor of growth. We also find that Oxyskyrin increases sensitivity of cells to radiation, and this effect is significant at radiation intensity lower than the standard intensity of cancer radiotherapy. On the basis of this study, Oxyskyrin shows promise for combined-modality cancer treatment.

Keywords: Oxyskyrin; Lecanora pseudistera; Murine SV40

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Introduction

Reinfection of tissue with cancer cells with acquired radio resistance during treatment is the grand challenge for cancer radiotherapy [1]. For this reason, radiotherapy is applied in combination with chemotherapy. The most effective of chemotherapeutic drug combinations inhibits growth of the cancer cell and also increases sensitivity of cancer cells to radiation. The radiosensitizing effect enhances radiotherapy at low radiation intensity. For this reason, radiotherapy in combination with chemotherapy (combined-modality treatment) is the best standard of care for most cancers [2]. However, the discovery rate of effective anti-cancer drugs is very slow [3]. We must turn to the secondary metabolites of the lichens as a domain of search for such compounds. This study explores the biological activity of Oxyskyrin, a secondary metabolite of the lichen *Lecanora pseudistera*.

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

Cancer is a complex disease that begins with the uncontrolled growth of the cell. The 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 the critical first step for drug discovery. In our method to determine the biological activity of Oxyskyrin, we test the effect on the growth of murine SV40 transformed bone blastoma cells in vitro. In addition, we test the effect in combination with irradiation with a range of intensity.

Materials and Methods

Chemicals: The chemical structure of Oxyskyrin 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 of cell culture to achieve the final concentrations of Oxyskyrin: 10 uM, 1 uM, 0.1 uM, 0.01 uM, 0.001 uM, and 0.0001 uM. The control group received 0.01 mL of growth medium.

Cells and cell culture

Murine SV40 transformed bone blastoma cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented

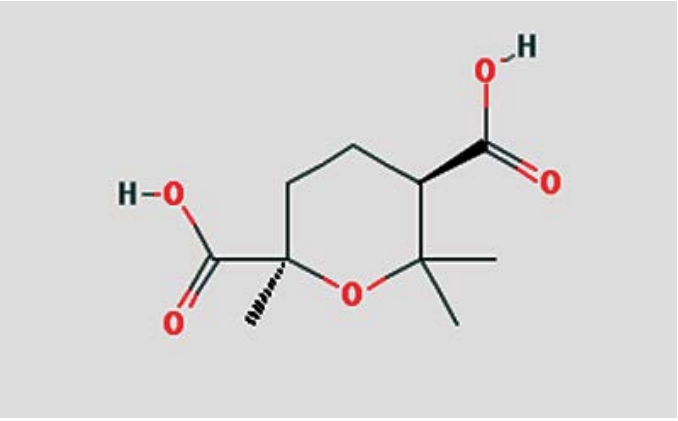

Figure 1: The structure of Oxyskyrin.

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 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. Oxyskyrin solutions were aliquoted to cells in 24- well plates. The treated cells were then cultured in 100-mm plastic tissue-culture dishes at 37 C with 5% CO_2 under high humidity. The final cell counts were measured after 5 days growth.

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.

Data analysis

Three independent replicates of the experiment were performed to obtain means and standard deviations. Mean cell counts were normalized to control cells grown in parallel.

Significance of differences between treatments were 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.

Results

Dose-dependent effect of Oxyskyrin on the growth of the rat glioblastoma cell

We cultured the cells in parallel with doses of Oxyskyrin at different concentrations. We measured the cell proliferation after 5 days in the logarithmic growth phase.

Below figureshows the results of the first experiment (Figure 2).

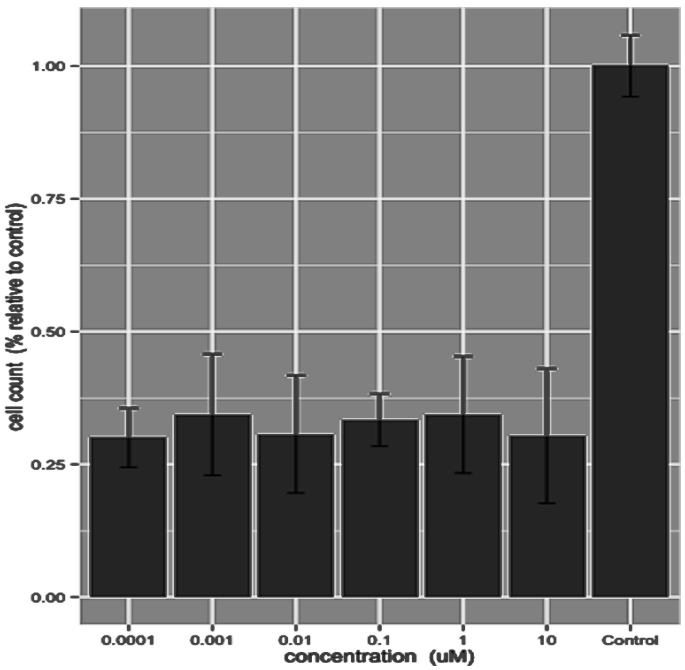

Figure 2: Dose-dependent effect of Oxyskyrin on the growth of murine SV40 transformed bone blastoma cells.

All concentrations of Oxyskyrin had a similar level of effect. And all concentrations cause a significant inhibition of cell growth compared to the control. Cell growth is inhibited with treatment at the lowest concentration of Oxyskyrin (0.0001 uM), which causes 70% slower proliferation compared to the control ($p<0.001$).

The X axis is concentration (uM) Oxyskyrin in culture tubes before growth. The Y axis is cell count after 5 days of growth, normalized to cell count of the control. Confidence intervals at 95% are indicated. The difference between 0.0001 uM Oxyskyrin treatment and control is significant ($p<0.001$).

Effect of Oxyskyrin in combination with irradiation on the growth of murine SV40 transformed bone blastoma cells

With the results of the first experiment, we test the lowest concentration Oxyskyrin (0.0001 uM) in combination with gamma radiation. We grow the cells identically as the first experiment, 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 of cell culture to achieve the final concentration of Oxyskyrin (0.0001 uM). The control group received 0.01 mL growth medium and no irradiation.

Below figure shows the results of the second experiment (Figure 3). Lower than nanomolar concentration of the Oxyskyrin powerfully enhances the inhibition effect of radiation on cell growth. This effect is significant at 0.5 Gy, the lowest level of radiation ($p=0.0012$).

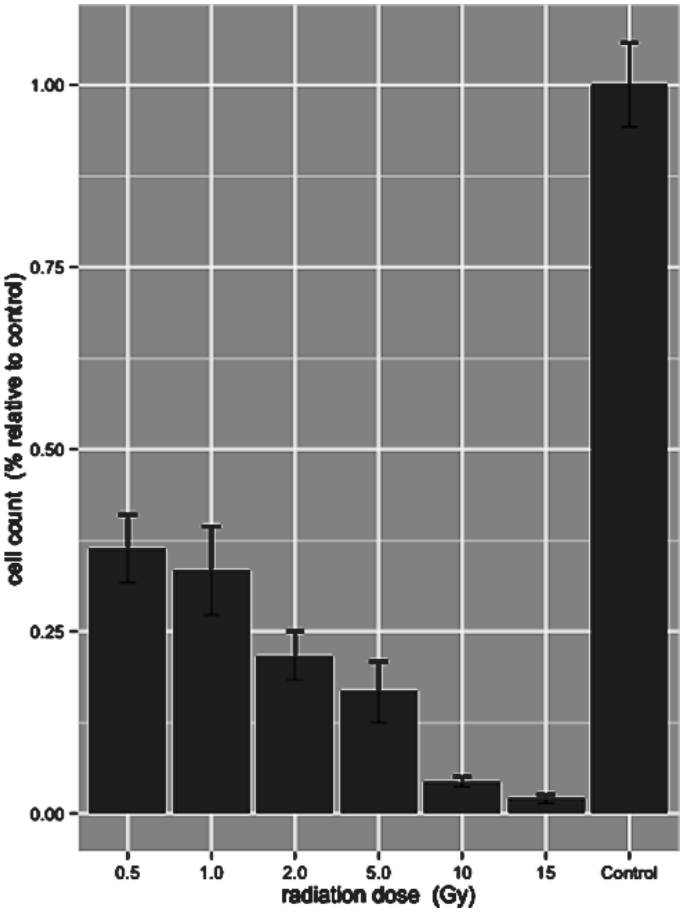

Figure 3: Effect of Oxyskyrin in combination with irradiation on the growth of murine SV40 transformed bone blastoma cells.

The X axis is intensity (Gy) of radiation. The Y axis is cell count after 5 days of growth, normalized to cell count of the control. Cells were irradiated after treatment with 0.0001 uM Oxyskyrin. Confidence intervals at 95% are indicated. The difference between 0.5 Gy and control is significant (p=0.0012).

Discussion

In this study, we test the biological activity of Oxyskyrin, secondary metabolite of the lichen *Lecanora pseudistera*. Specifically we measure the effect on growth of murine SV40 transformed bone blastoma cells in vitro.

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

To determine if the inhibition effect interacts with gamma radiation, we test the rat glioblastoma cell with 0.0001 uM Oxyskyrin and a range of radiation intensity. The result proves that Oxyskyrin is also a radiosensitizer. Oxyskyrin enhances the inhibition effect of radiation on the growth of cancer. This effect is significant at 0.5 Gy, a radiation dose that is lower than the standard radiation dose in cancer radiotherapy.

We propose the biological activity of Oxyskyrin is related to lichen ecology. It is known that lichens are adapted forthe 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 Oxyskyrin is a radiosensitizer with anti-cancer activity. In the next step, we will prove that Oxyskyrin is effective against cancer in animal and human. We conclude that Oxyskyrin is a promising new drug for the combined-modality treatment of cancer.

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