Medicinal chemistry

Idandah et al., Med chem 2013, 3:3
http://dx.doi.org/10.4172/2161-0444.1000145

Research Article Open Access

7-Chloronorlichexanthone Inhibits the Growth of Murine SV40 Transformed Lymphoid Sarcoma Cells *in vitro*

Alanguay C Idandah*, Bocoo Y Akechowoo, Nanayforrah HO Oloosamah, and Shollahday D Labolonee

Serjay International College, Conakry, Guinea

Abstract

We test the effects of 7-Chloronorlichexanthone, secondary metabolite of the lichen *Biatora ru-betaula*, on the growth of murine SV40 transformed lymphoid sarcoma cells *in vitro*. We find that 7-Chloronorlichexanthone is a potent inhibitor of growth. We also find that 7-Chloronorlichexanthone 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, 7-Chloronorlichexanthone shows promise for combined-modality cancer treatment.

Keywords: Lymphoid sarcoma cells; Murine SV40; *In vitro*

Introduction

Reinfection of tissue with cancer cells with acquired radioresistance 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 7-Chloronorlichexanthone, a secondary metabolite of the lichen *Biatora ru-betaula*.

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 7-Chloronorlichexanthone, we test the effect on the growth of murine SV40 transformed lymphoid sarcoma 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 7-Chloronorlichexanthone 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 7-Chloronorlichexanthone: 10 µM, 1 µM, 0.1 µM, 0.01 µM, 0.001 µM, and 0.0001 µM. The control group received 0.01 mL of growth medium.

Cells and cell culture

Murine SV40 transformed lymphoid sarcoma cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mg/ml N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 100 µ/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. 7-Chloronorlichexanthone 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.

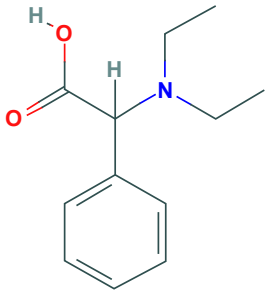

Figure 1: The structure of 7-Chloronorlichexanthone.

***Corresponding author:** Alanguay C Idandah, Serjay International College,Conakry, Guinea, Tel: + 224 442 020; E-mail: kuumwa@yahoo.com, kuumwa@afra-mail.com

Received April 22, 2013; **Accepted** July 25, 2013; **Published** July 27, 2013

Citation: Idandah AC, Akechowoo BY, Oloosamah NHO, Labolonee SD (2013) 7-Chloronorlichexanthone Inhibits the Growth of Murine SV40 Transformed Lymphoid Sarcoma Cells *in vitro*. Med chem 3: 238-240. doi:10.4172/2161-0444.1000145

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 7-Chloronorlichexanthone on the growth of the rat glioblastoma cell

We cultured the cells in parallel with doses of 7-Chloronorlichexanthone 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 7-Chloronorlichexanthone 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 7-Chloronorlichexanthone (0.0001 μM), which causes 70% slower proliferation compared to the control (p<0.001).

Effect of 7-Chloronorlichexanthone in combination with irradiation on the growth of murine SV40 transformed lymphoid sarcoma cells

With the results of the first experiment, we test the lowest concentration 7-Chloronorlichexanthone (0.0001 μM) 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 7-Chloronorlichexanthone (0.0001 μM). The control group received 0.01 mL growth medium and no irradiation.

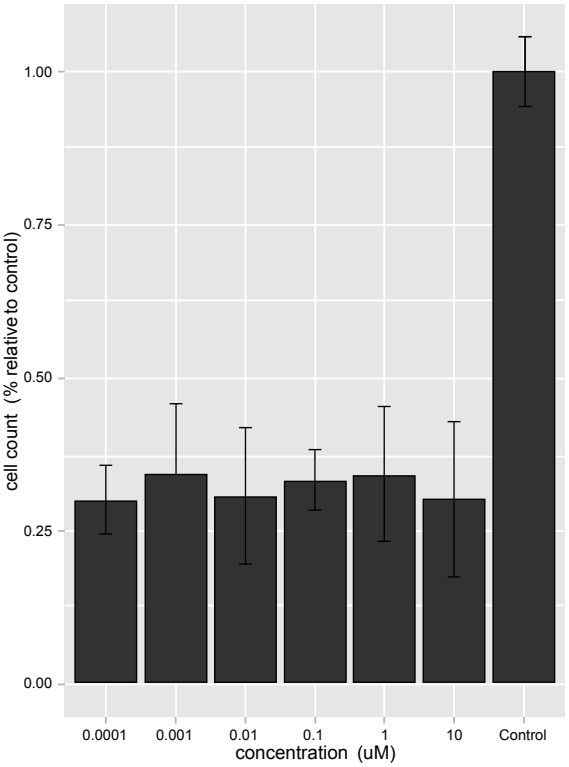

Figure 2: Dose-dependent effect of 7-Chloronorlichexanthone on the growth of murine SV40 transformed lymphoid sarcoma cells. The X axis is concentration (uM) 7-Chloronorlichexanthone in culture tubes before growth. The Y axis is cell count after 5 days of growth, normalized to cell count of the control. Confidence intervals at 95% are indicated. The difference between 0.0001 uM 7-Chloronorlichexanthone treatment and control is significant (p<0.001).

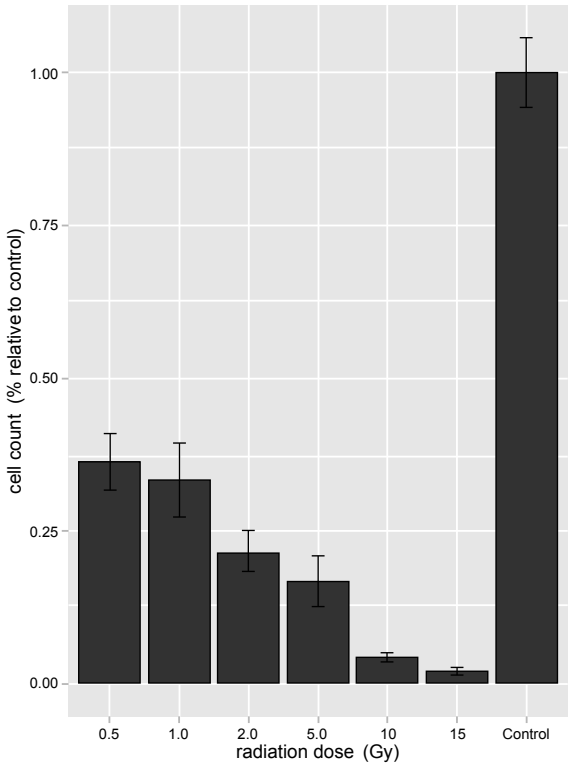

Figure 3: Effect of 7-Chloronorlichexanthone in combination with irradiation on the growth of murine SV40 transformed lymphoid sarcoma cells. The X axis is intensity (Gy) of radiation. The Y axis is cell count after 5 days of growth, normalized to cell count of the control. Cells were irradiated after treatment with 0.0001 uM 7-Chloronorlichexanthone. Confidence intervals at 95% are indicated. The difference between 0.5 Gy and control is significant (p=0.0012).

Figure 3 shows the results of the second experiment. Lower than nanomolar concentration of the 7-Chloronorlichexanthone 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).

Discussion

In this study, we test the biological activity of 7-Chloronorlichexanthone, secondary metabolite of the lichen *Biatora ru-betaula.* Specifically we measure the effect on growth of murine SV40 transformed lymphoid sarcoma cells *in vitro.*

Our results show that 7-Chloronorlichexanthone inhibits cell growth. The mechanism of action is unknown, but the effect is potent. Even at the lowest dose (0.0001 μM), 7-Chloronorlichexanthone 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 μM 7-Chloronorlichexanthone and a range of radiation intensity. The

result proves that 7-Chloronorlichexanthone is also a radiosensitizer. 7-Chloronorlichexanthone 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 7-Chloronorlichexanthone 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 7-Chloronorlichexanthone is a radiosensitizer with anti-cancer activity. In the next step, we will prove that 7-Chloronorlichexanthone is effective against cancer in animal and human. We conclude that 7-Chloronorlichexanthone is a promising new drug for the combined-modality treatment of cancer.

Acknowledgements

This work was supported by a graduate thesis research grant for Bocoo Y. Akechowoo. We thank Dan N. Raboniras for help obtaining chemicals and Kobi V. Chananov for helpful comments.

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