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Norstictic Acid Inhibits the Growth of Murine SV40 Transformed Lymphoid Carcinoma Cells *In vitro*

Bangolah I.E. Affeam, Shellaleh L.N. Alloula and Accomodo O.Q. Semeyannah
Lahtokay Normal University, Ouagadougou, Burkina Faso

Abstract: Researchers test the effects of Norstictic acid, secondary metabolite of the lichen *Buellia zahlbruckneri*, on the growth of murine SV40 transformed lymphoid carcinoma cells *in vitro*. Researchers find that Norstictic acid is a potent inhibitor of growth. Researchers, also find that Norstictic acid increases sensitivity of cells to radiation and this effect is significant at a radiation intensity lower than the standard intensity of cancer radiotherapy. On the basis of this study, Norstictic acid shows promise for combined-modality cancer treatment.

Key words: Norstictic acid, cancer, growth, radiotherapy, *in vitro*, *Buellia zahlbruckneri*

INTRODUCTION

Reinfection of tissue with cancer cells with acquired radioresistance during treatment is the grand challenge for cancer radiotherapy (Baumann *et al*., 2008). 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 (Prestwich *et al*., 2007). However, the discovery rate of effective anti-cancer drugs is very slow (Kamb *et al*., 2007). Researchers must turn to the secondary metabolites of the lichens as a domain of search for such compounds. This study explores the biological activity of Norstictic acid, a secondary metabolite of the lichen *Buellia zahlbruckneri*.

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. About >700 secondary metabolites of lichens are isolated but only a small number are characterized for biological activity (Boustie and Grube, 2005).

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 (Vermeulen *et al*., 2003).

Therefore, the establishment of the inhibition of proliferation of cancer cells *in vitro* is the critical 1st step for drug discovery. In the method to determine the biological activity of Norstictic acid, researchers test the effect on the growth of murine SV40 transformed lymphoid carcinoma cells *in vitro*. In addition, researchers test the effect in combination with irradiation with a range of intensity.

MATERIALS AND METHODS

Chemicals: The chemical structure of Norstictic acid is shown in Fig. 1. Pure extracts were dissolved and serially diluted in a 2:1 mixture of ethanol and phosphate buffered

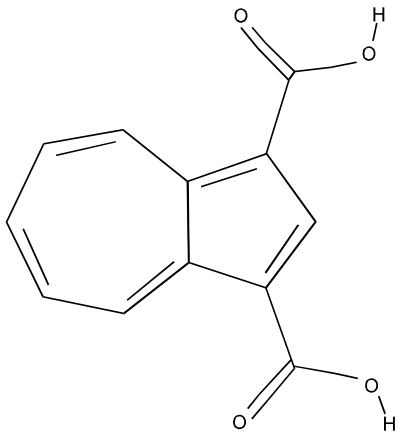

Fig. 1: The structure of Norstictic acid

Corresponding Author: Bangolah I.E. Affeam, Lahtokay Normal University, Ouagadougou, Burkina Faso

saline (EtOH/PBS, pH 7.4). These solutions were added as aliquots of 0.01-0.99 mL of cell culture to achieve the final concentrations of Norstictic acid: 10, 1, 0.1, 0.01, 0.001 and 0.0001 uM. The control group received 0.01 mL of growth medium.

Cells and cell culture: Murine SV40 transformed lymphoid carcinoma cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mg mL^{-1} N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid, 100 U mL^{-1} penicillin G, 0.1 mg mL^{-1} streptomycin, 2 mg mL^{-1} 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. Norstictic 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°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 0.5-15 Gy were used. The dose rate was 1 Gy/4 sec. A control group received no radiation?

Data analysis: The 3 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 $p<0.01$ was accepted as significant.

RESULTS AND DISCUSSION

Dose-dependent effect of Norstictic acid on the growth of the rat glioblastoma cell: Researchers cultured the cells in parallel with doses of Norstictic acid at different concentrations. Researchers measured the cell proliferation after 5 days in the logarithmic growth phase. Figure 2 shows the results of the first experiment. All concentrations of Norstictic acid 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 Norstictic acid (0.0001 uM) which causes 70% slower proliferation compared to the control ($p<0.001$).

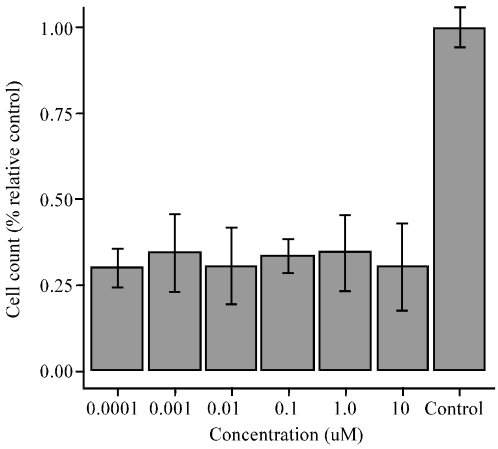

Fig. 2: Dose-dependent effect of Norstictic acid on the growth of murine SV40 transformed lymphoid carcinoma cells. The x-axis is concentration (uM) Norstictic acid in culture tubes before growth. The y-axis is cell count after 5 days of growth, normalized to cell count of the control. Confidence intervals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

Effect of Norstictic acid in combination with irradiation on the growth of murine SV40 transformed lymphoid carcinoma cells: With the results of the first experiment, researchers test the lowest concentration Norstictic acid (0.0001 uM) in combination with gamma radiation. Researchers grow the cells identically as the 1st 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 0.01-0.99 mL of cell culture to achieve the final concentration of Norstictic acid (0.0001 uM). The control group received 0.01 mL growth medium and no irradiation.

Figure 3 shows the results of the 2nd experiment. Lower than nanomolar concentration of the Norstictic acid powerfully enhances the inhibition effect of radiation on cell growth. This effect is significant at 0.5 Gy, the lowest level of radiation ($p = 0.0012$).

In this study, researchers test the biological activity of Norstictic acid, secondary metabolite of the lichen *Buellia zahlbruckneri*. Specifically, measure the effect on growth of murine SV40 transformed lymphoid carcinoma cells *in vitro*.

The results show that Norstictic acid inhibits cell growth. The mechanism of action is unknown but the effect is potent. Even at the lowest dose (0.0001 uM),

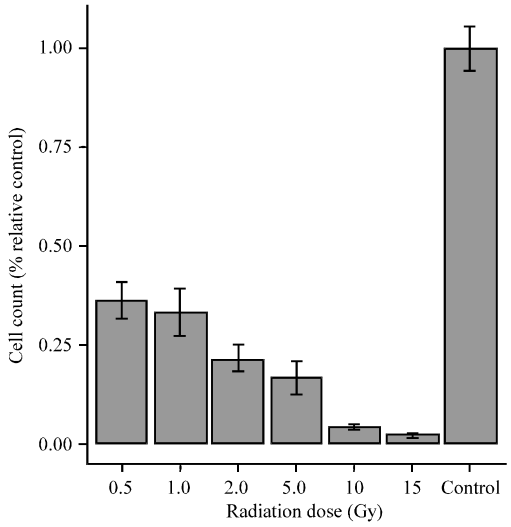

Fig. 3: Effect of Norstictic acid in combination with irradiation on the growth of murine SV40 transformed lymphoid carcinoma cells. The x-axis is intensity (Gy) of radiation. The y-axis is cell count after 5 days of growth, normalized to cell count of the control. Cells were irradiated after treatment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

Norstictic acid 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, researchers test the rat glioblastoma cell with 0.0001 uM Norstictic acid and a range of radiation intensity. The result proves that Norstictic acid is also a radiosensitizer. Norstictic acid 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.

Researchers propose the biological activity of Norstictic 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 (Lawrey, 1986). Therefore, it is not surprising that the secondary metabolites of the lichen can enhance the effect of radiation and inhibit foreign cells.

CONCLUSION

The study is the first to demonstrate that Norstictic acid is a radiosensitizer with anti-cancer activity. In the next step, researchers will prove that Norstictic acid is effective against cancer in animal and human. It is concluded that Norstictic acid is a promising new drug for the combined-modality treatment of cancer.

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