The Effects of Duocarmycin SA and Proton Radiation on Glioblastoma Cells in Vitro

Nabhan Jankeel, PharmD Candidate 2021, Dale L. Boger, PhD, Marcelo Vazquez, MD, PhD, Kristopher Boyle, PhD
LLU School of Pharmacy, LLUMC Radiation Medicine Department, Loma Linda, CA, The Scripps Research Institute, La Jolla, CA

Background

Glioblastoma multiforme (GBM) is the most common and the most aggressive form of primary brain cancer, with patient median survival of 15–18 months. Because GBM tumor cells are highly infiltrative throughout the brain at diagnosis, complete removal of all tumor cells by surgery is not possible and disease recurrence following tumor resection results. For this reason, surgery is followed by treatment with radiation and chemotherapy in GBM patients.

Objective

To evaluate the cellular radiobiological effects of proton irradiation in combination with duocarmycin SA (DSA) in order to establish the scientific rationale for future animal studies and the ultimate clinical implementation of proton radio-chemotherapy.

Introduction

Proton therapy is considered the most effective form of radiation therapy for GBM. The most frequently used drugs in the treatment of GBM are members of the DNA-alkylator class, such as carmustine (BCNU) and temozolomide (TMZ). While clinically useful, TMZ is a fairly impotent and ineffective compound. The duocarmycin class of antitumor antibiotics, exemplified by duocarmycin SA (DSA), which is isolated from Streptomyces bacteria, is an exceptionally potent group of agents capable of inducing a sequence-selective alkylation of duplex DNA.

Methods

Experiments were performed using the human glioblastoma cancer cell line:

1. LN-18 glioblastoma obtained from ATCC

Endpoints:

1. Cell Proliferation Assay

Cells were harvested and stained with Trypan Blue viability test (Molecular Probes®) 72 h. post-treatment and counted using a Countess® II Automated Cell Counter.

2. Colony Formation Assay

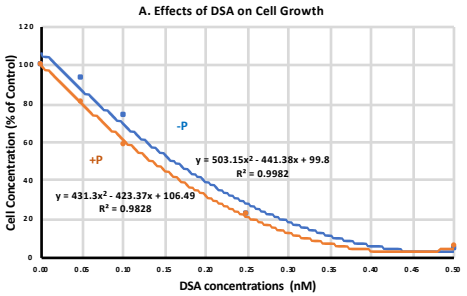
Cells were plated for survival analysis using the clonogenic assay technique of Puck and Marcus. In brief, the cells were incubated at 37 °C for 2 weeks to allow surviving cells to form colonies, which were ultimately stained and counted. Colonies consisting of more than 50 cells were scored as survivors. IC values were obtained using a linear quadratic model (LQM) and multi-target model.

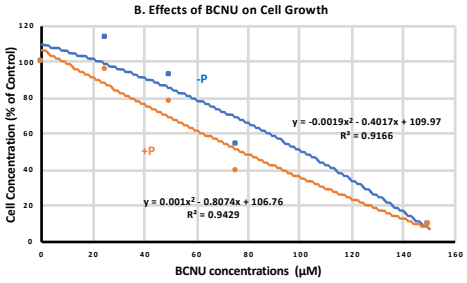
Treatment:

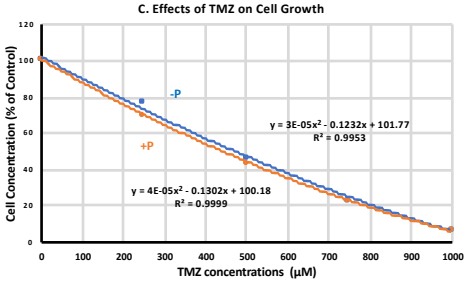
LN-18 cells were dosed with drug an hour before treatment with irradiation:

A. Duocarmycin SA (DSA: 0.001–0.50 nM, proton irradiation: 3 Gy)
B. Carmustine (BCNU: 10–150 µM, proton irradiation: 3 Gy)
C. Temozolomide (TMZ: 100–1000 µM, proton irradiation: 3 Gy)
D. Proton irradiation: 250 MeV, dose: 3 Gy

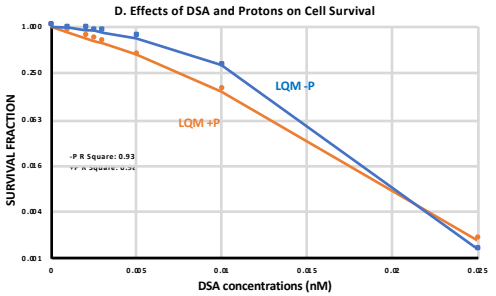
Cell Proliferation Assay Results

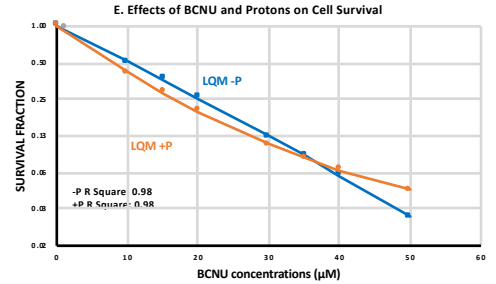


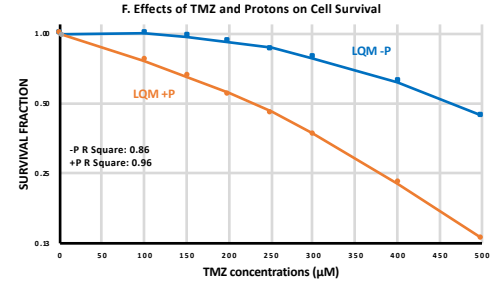




Colony Formation Assay Results







	A. Cell Proliferation Assay		B. Colony Formation Assay	
Drug	IC50	IC37	D50	D37
DSA	0.147 nM	0.208 nM	0.00291 nM	0.0042 nM
DSA +P	0.124 nM	0.179 nM	0.00285 nM	0.00411 nM
TMZ	465.9 µM	619.0 µM	560.5 µM	808.56 µM
TMZ +p	445.8 µM	593.5 µM	186.2 µM	268.6 µM
BCNU	94.6 µM	101.0 µM	9.78 µM	14.11 µM
BCNU +p	72.7 µM	117.0 µM	10.2 µM	14.8 µM

Table 1: Summary of toxicity parameters for cells using different drugs in the presence and absence of protons

Discussion

Table 1 summarizes the toxicity parameters for LN-18 cells exposed to different drugs in the presence and absence of protons. The extraordinary potency of DSA was observable in the LN-18 glioblastoma cell line, as evidenced by the nearly 200 million-fold difference in potency compared to TMZ for D50 in the colony formation assay. Based on the results of these experiments, one can conclude that both DSA and BCNU show additive effects when combined with proton irradiation in both assays employed, but neither drug demonstrates a synergistic effect. For example, in figure D, comparing the D50 and D37 without protons to D50 or D37 with protons, the difference between their values (0.00291 nM and 0.00285 nM for D50, or 0.0042 nM to 0.00411 nM) is not synergistic. For DSA, the most likely explanation is that high concentrations of DSA wash out the effect of a modest dose of radiation. The same conclusion may be applicable to BCNU.

However, synergism was observed for TMZ in combination with proton radiation in the colony formation assay. No synergism was observed in the more short-term cell proliferation assay. As shown in table 1B, TMZ without proton irradiation has a value of 560.5 µM for D50, whereas in the presence of proton irradiation this value changed significantly to 186.2 µM. Thus, TMZ in combination with proton irradiation demonstrated synergism. This is probably due to the fact that TMZ is fairly impotent, and so the addition of proton irradiation significantly increases the overall cytotoxic effect of a modest dose of TMZ.

Previous experiments using a constant concentration of DSA (0.001 nM) and escalating doses of proton irradiation in a different cell line (U-138 glioblastoma) showed a significant synergistic effect[1]. We find it likely that a similar synergism will be observed with LN-18 cells under identical conditions using DSA.

Conclusion

DSA did not demonstrate synergism with proton irradiation against LN-18 glioblastoma cells under the current experimental conditions. Nonetheless, the extraordinary potency of DSA in the colony formation assay is a promising initial result, and synergism with proton irradiation will be investigated using a constant 0.001 nM concentration of DSA with escalating doses of proton radiation. A comparison of these experiments using DSA to similar experiments employing TMS and BCNU will be reported in due course.

References

[1]Boyle, K., Boger, D., Wroe, A., & Vazquez, M. (2018). Duocarmycin SA, a potent antitumor antibiotic, sensitizes glioblastoma cells to proton radiation. Bioorganic & Medicinal Chemistry Letters, 28(16), 2688-2692.

Acknowledgments

We want to thank Loma Linda University School of Pharmacy for its financial support, LLUMC Radiation Medicine Department for making available the proton facility, and Professor Dale Boger for invaluable advice and supplying all DSA used in these experiments.