Gallium Maltolate as Treatment for Pediatric Glioma

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ABSTRACT

Pediatric high-grade gliomas are devastating central nervous system tumors with a poor prognosis. These tumors become rapidly resistant to conventional therapy, including, chemotherapy or radiation therapy. Subsequent treatment options are limited. There is thus a dire need to develop novel treatments for these cancers.

We and others have shown that adult glioblastoma cells have a high requirement for iron to support their growth and viability. We have reported gallium compounds interact with critical iron-dependent proteins in cancer cells to inhibit mitochondrial function and the iron-dependent activity of ribonucleotide reductase. Our recent studies show that gallium tumor rat model. There is now an increasing interest in determining the potential of GaM as therapy for pediatric brain tumors. In addition to our work, studies of GaM have been initiated by other investigators (L. Bernstein, PhD, personal communication). Herein, we present our results of preliminary investigations to explore the potential antineoplastic activity of GaM in pediatric gliomas. Pediatric glioma cell lines representing three glioblastoma (SJ-GBM2, SF188, CHLA-200) and two ATRT (CHLA-266 and CHLA-02) cell lines were incubated for 48 – 144 h with increasing concentrations of GaM and the effect on cell proliferation was determined by MTT and PE assays. In CHLA-200, SF188, and SJ-GBM2 cells, the IC50 of GaM was 48.4 µM, 73.9 µM and 100 µM, respectively, while in CHLA-266 ATRT cells the IC50 was 16.3 µM after a 96h incubation of cells with GaM. Annexin V staining showed GaM induces apoptosis in GBM and ATRT cells. Our results show that GaM inhibits the growth of different pediatric gliomas with varying efficacy. Further studies are in progress to investigate the differences in iron metabolism among the pediatric glioma cells, drug combinations of GaM with other standard therapeutic agents, and the molecular basis for drug resistance.

Pediatric Cell Lines Studied

- CHLA-02-ATRT: Homo sapiens, Atypical Teratoid and Rhabdoid Tumor, from 1.7 years old male. Suspension; single cells or clusters.
- CHLA-266-ATRT: Homo sapiens, Atypical Teratoid and Rhabdoid Tumor, from 2.5 years old female. Adherent; single cells or clusters.
- CHLA-200-GBM: Homo sapiens, Glioblastoma multiforme, from 12 years old male. Adherent; single cells or clusters.
- SF188-GBM: Homo sapiens, Glioblastoma multiforme, from 12 years old male. Adherent; single cells or clusters.
- SJ-GBM2: Homo sapiens, Glioblastoma multiforme, from 41 years old female. Adherent cells.

Growth Rate of Pediatric Cell Lines

Growth rate of SJ-GBM2 and CHLA-266 cell lines were measured after 24, 48, 72 and 96 hours for viability after addition of different concentrations of gallium maltolate (GaM). Cell viability was assessed by MTT assay. Each data point is the result of the average of 4 technical replicates. The experiments was repeated at least three times. GaM concentrations of <100 µM were not cytotoxic.

Effect of Gallium Maltolate on the Growth of Human Brain Microvascular Endothelial Cells

Human brain microvasculature endothelial cells were monitored after 24, 48, 72 and 96 hours for viability after addition of different concentrations of gallium maltolate (GaM). Cell viability was assessed by MTT assay. Each data point is the result of the average of 4 technical replicates. The experiments was repeated at least three times. GaM concentrations of <100 µM were not cytotoxic.

Effect of GaM on pediatric tumor cell line viability

Effect of gallium maltolate on Lysosomes

Induction of Apoptosis by gallium maltolate

Conclusions and Future Directions

- GaM inhibits the growth of pediatric ATRT and glioma cells and induces apoptosis in vitro in both a time and concentration-dependent manner.
- GaM cytotoxicity may involve action on lysosomal function.
- Further studies are planned to evaluate the antineoplastic activity in pediatric brain tumors in animal models.
- GaM may have potential therapeutic application in the treatment of pediatric brain tumors.

References


Previous studies have suggested that radiogallium (Ga-67) traffics to the lysosomal compartment. To investigate this in pediatric glioma cells, we treated CHLA-266 cells with GaM and then stained for lysosomal-Associated Membrane Protein-1 (LAMP-1). Immunofluorescence studies with LAMP-1 antibody with counterstaining using DAPI (nuclear staining) were conducted. After 24 hours of incubation with GaM, lysosomes in cells displayed a stronger LAMP-1 signal than control cells. AIM software was used for image profile analysis; this program plots the intensity of the fluorophore. As shown in the figure, the intensity of the LAMP-1 signal is greater in GaM-treated cells with respect to the control cells. Picture were taken with confocal microscopy, 60X magnification.