Gallium Maltolate as Treatment for Pediatric Glioma

Salvatore Molino[1], Mona M. Al-Gizawiy[2] Jeffrey Knipstein[3], Kathleen M. Schmainda[2,4], Christopher R. Chitambar[1,2]

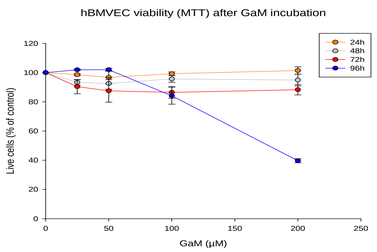
[1] Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[2] Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[3] Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA
[4] Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

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 maltolate (GaM), a novel metallocompound (currently being evaluated clinically) inhibits the growth of glioblastoma *in vitro* and *in vivo* in an adult orthotopic U87 xenograft brain 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.

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

Pediatric Cell Lines Studied

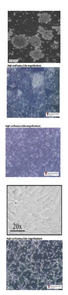
CHLA-02-ATRT: Homo sapiens, Atypical Teratoid and Rhabdoid Tumor, from 1.7 years old male. Suspension; single cells or clusters.

CHLA-266-ATRT: Homo sapiens, Atypical Teratoid and Rhabdoid Tumor, from 2.5 years old female. Adherent; single cells or clusters.

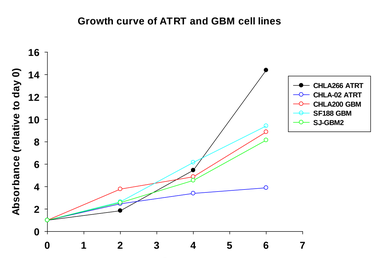
CHLA-200-GBM: Homo sapiens, Glioblastoma multiforme, from 12 years old male. Adherent cells.

Sf-188-GBM: Homo sapiens, Glioblastoma multiforme, from 12 years old male. Adherent cells.

SJ-GBM2: Homo sapiens, Glioblastoma multiforme, from 4.1 years old female. Adherent cells.



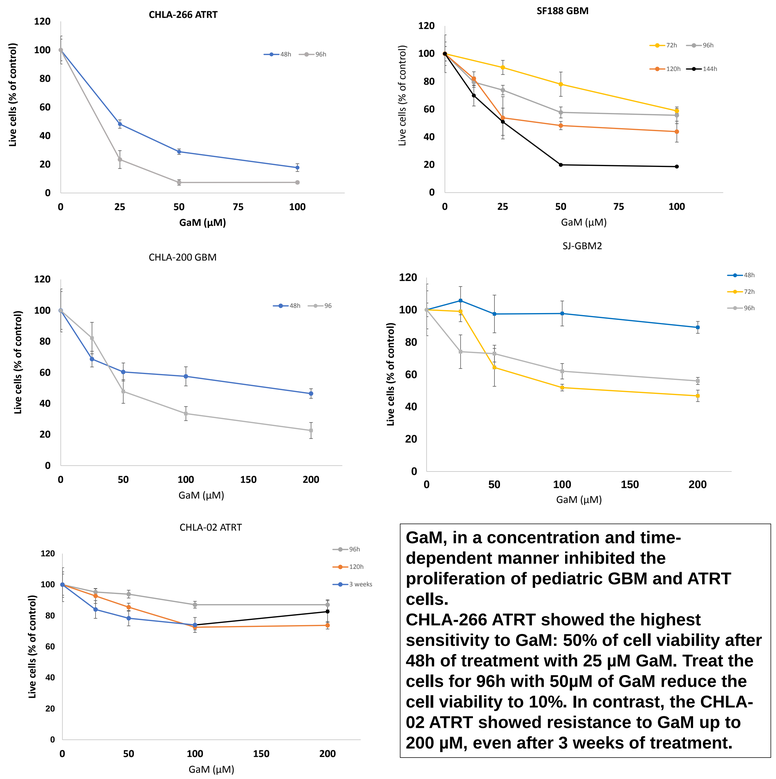
Growth Rate of Pediatric Cell lines



Pediatric GBM and ATRT cells were starved in culture medium without FBS in order to be synchronized and to compare their doubling time.

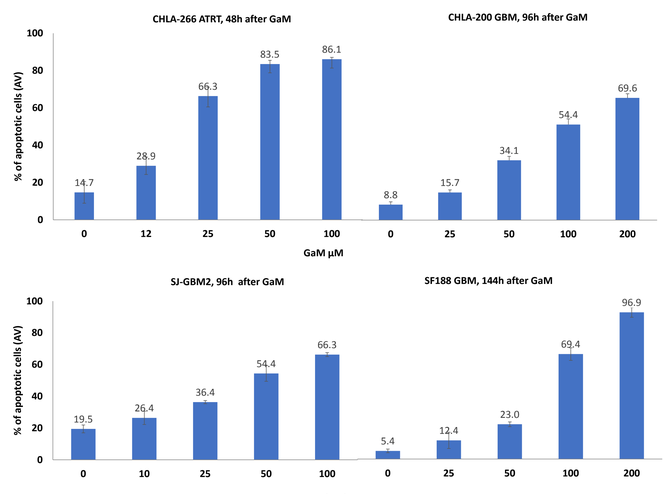
Cells	Doubling Time
CHLA-266 ATRT:	46h
CHLA-02 ATRT:	60h
CHLA-200 GBM:	40h
SF-188 GBM:	30h
SJ-GBM2:	30h

Effect of GaM on pediatric tumor cell line viability



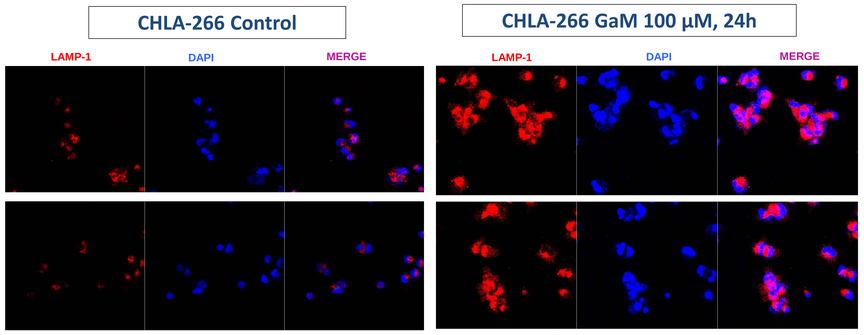
GaM, in a concentration and time-dependent manner inhibited the proliferation of pediatric GBM and ATRT cells.
CHLA-266 ATRT showed the highest sensitivity to GaM: 50% of cell viability after 48h of treatment with 25 μM GaM. Treat the cells for 96h with 50μM of GaM reduce the cell viability to 10%. In contrast, the CHLA-02 ATRT showed resistance to GaM up to 200 μM, even after 3 weeks of treatment.

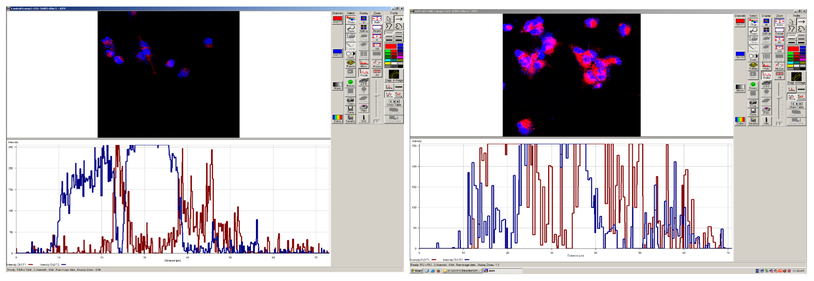
Induction of Apoptosis by gallium maltolate



To examine whether the growth inhibitory effects of GaM were due to induction of apoptosis, GBM and ATRT cells were incubated with different concentration of GaM and stained with annexin V and PI to monitor apoptosis level by flow cytometry. GaM induced apoptosis at different level in all the cells analyzed; In CHLA-266, after 48h of incubation, 25 μM of GaM is sufficient to induce apoptosis in 66% of cells population.

Effect of gallium maltolate on Lysosomes





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.

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

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