CANCER RESEARCH

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# Abstract 4176: Involvement of the lysosome in the cytotoxicity of Gallium Maltolate in human pediatric brain tumor cell lines

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## Abstract

Introduction: Gallium maltolate (GaM) is a novel small molecule metallocompound that acts as an iron mimetic Trojan Horse to disrupt iron-dependent proteins essential in tumor biology. We recently reported that GaM retards the growth of orthotopically implanted adult human glioblastoma (GBM) xenografts in a rodent brain tumor model and is cytotoxic to glioblastoma stem cells (Mol Cancer Ther 17:1240, 2018). GaM's mechanisms of action include inhibition of both mitochondrial function and iron-dependent ribonucleotide reductase. In the present study, we further explored whether GaM has antitumor activity in pediatric brain tumor cells.

Experimental procedures: The cytotoxicity of GaM in pediatric GBM and Atypical Teratoid Rhabdoid Tumor (ATRT) cell lines was examined by MTT and clonogenic assays. Apoptotic cell death was assessed by flow cytometry with annexin V (AV) and propidium iodide (PI) staining. Cellular bioenergetics was measured using a Seahorse XF96 Extracellular Flux Analyzer. GaM interaction with lysosomes was analyzed by immunofluorescence and electron microscopy (EM).

Results: In a panel of pediatric brain tumor cell lines, CHLA-266 ATRT and GBM cells displayed the greatest sensitivity to GaM. Complete inhibition of colony growth was achieved with 6 - 7 μM GaM over a 96-hour incubation. With a shorter incubation of 48-h, 25 μM GaM induced apoptosis in > 60% of CHLA-266 ATRT cells. In contrast, a longer incubation of 96-h with 25 μM GaM was required to induce apoptosis in > 50% of CHLA-200 GBM, 69% of SF188 GBM and 60% of SJ-GBM2 cell lines. After a 24-h of incubation with GaM, prior to detectable cell death, CHLA-266 and GBM cells displayed a marked reduction in mitochondrial reserve capacity, glycolysis, and glycolytic capacity. Immunofluorescence staining for the lysosomal marker LAMP1 showed that GaM produced an increase in lysosome size and number after a 24-h or 48-h incubation relative to control cells. EM studies demonstrated a gallium signal within the lysosomal compartment as early as 6-h of incubation. EM findings consistent with GaM-induced apoptosis were noted after a 24-h incubation, thus confirming the results of the AV/PI assay. After 24-h however, gallium was no longer detectable in the lysosomes, suggesting that lysosomal disruption led to the release of gallium into the cytoplasm.

Conclusions: Our results identify GaM as a novel compound with significant activity against a panel of cell lines representative of pediatric brain tumors. Most of the cell lines used in our study were derived from tumors taken from heavily pre-treated patients and were resistant to conventional chemotherapeutic drugs. Their sensitivity of GaM suggests that GaM does not share cross-resistance with other antineoplastic drugs. Our study also provides new insight into the lysosomes as a target for gallium. The prognosis of adult and pediatric brain tumors is dismal and there is an urgent need to develop newer drugs for this malignancy. Further investigation of GaM as a therapeutic agent for brain tumors is warranted.

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