Intranasal perillyl alcohol (POH)induces endoplasmic reticulum stress (ERS) in temozolomide sensitive and resistant malignant gliomas

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Introduction

Perillyl alcohol (POH) is a monoterpene that has been used orally for the treatment of systemic cancer. Due to significant gastrointestinal side effects and lack of overall efficacy, this agent was not used as an oral anti-cancer agent. Recently, POH was administered intranasally in a Phase II trial for the treatment of recurrent malignant gliomas, with 50% progression free survival (PFS) for 6 months. The present study further explores the effects and mechanisms of POH on temozolomide (TMZ) sensitive/resistant gliomas in vitro/in vivo.

Materials and Methods

Measurements of Endoplasmic Reticulum (ER)was determined by western blots of ERS markers. Invasion assay performed using Boyden chambers. Intranasal delivery of POH was conducted in nude mice. Cells and Reagents: The human glioma cell lines were used: U87, U251, A172. The TMZ-resistant glioma cells were prepared by treating the tumor cell lines with increasing concentrations of TMZ ranging from 10 to 100 μM over a period of 2-3 months. Cells were labeled with renilla luciferase (RL). Glioma tumor cells were cultured in DMEM supplemented with 10% FBS, penicillin, streptomycin in a 5% CO2 humidified incubator at 37°C. Pharmaceutical grade POH was synthesized by NORAC, Inc. Cytotoxicity Method:. Standard MTT assay Colony forming assay (CFA): Cells were treated with drugs for 48 hours, washed, and then cultured on agar for 11 days. Colonies were quantified. Cytokine analysis: Interleukin -8 (IL-8), and vascular endothelial growth factor (VEGF) were evaluated using ELISA kits. In-Vivo animal experiment:
The intracranial xenograft nude mouse model was used. Luciferase labeled cells (2x105) were implanted intracranially; 7 days later treatment was begun. POH was administered intranasally in a volume of 20 μl, and diluted in DMSO. Tumors were imaged weekly, and intensity quantified. Statistical Analysis: Significance was evaluated using the Student's two-tailed t-test; $p < 0.05$ *, $p<0.01$ **.

Results

POH induced apoptosis in TMZ-sensitive and TMZ-resistant glioma cells. This agent induces cytotoxicity via ER stress pathway as shown by the increased expression of glucose-regulated protein-78 (GRP78), activating transcription factor 3 (ATF3), and C/EBPhomologous protein (CHOP). Combination with other ERS inducing agents ie 2,5-dimethyl-celecoxib (DMC) or nelfinavir (NFV) accentuated this effect. POH also decreased the invasive capacity of sensitive and resistant glioma cells. To demonstrate whether IN delivery of POH is effective in treating gliomas, animals bearing intracranial tumors were treated intranasally with POH. Luciferase positive U251 TMZ-resistant cells were stereotactically implanted into the right frontal lobe of nude mice. The intranasal delivery of POH had no toxicity, and demonstrated a decrease in tumor growth,increase in survival, and increased GRP78 in tumor tissue.

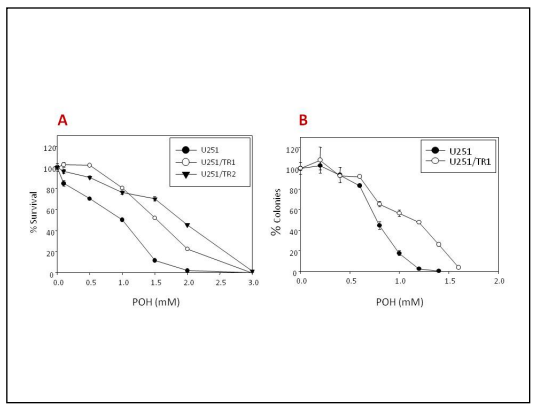

Fig 1.POH is cytotoxic for TMZ-sensitive and TMZ-resistant U251 cells. (A) Comparison of cytotoxicity of POH in U251 TMZ-sensitive and TMZ-resistant (U251TR) cells using the MTT assay. (B) Comparison using CFA in U251 sensitive and resistant cells.

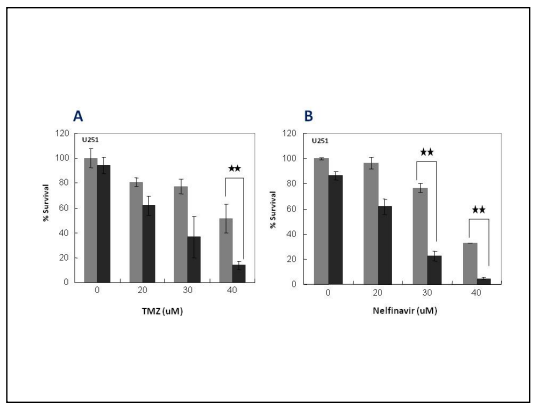

Fig. 2 POH acts as a chemosensitizing agent. U251 cells were treated for 48 hrs with POH (0.6mM) and different drugs: TMZ (A),and Nelfinavir (B) U251

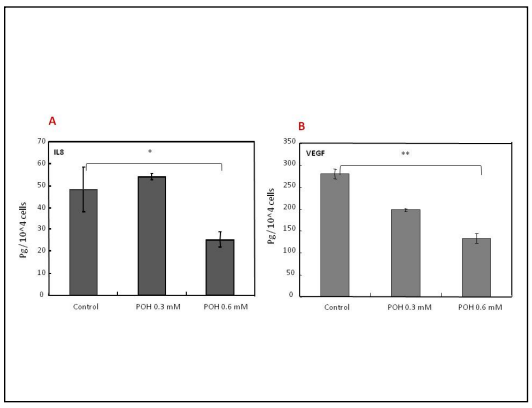

Fig. 3 POH reduces cytokine production by glioma cells . Conditioned media from U87 cells treated with POH was tested for pro-angiogenic growth factors (A) IL-8; and (B) VEGF

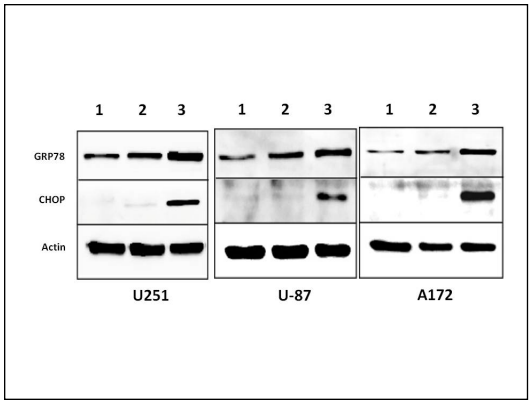

Fig. 4. POH mechanism of action. ER Stress markers (GRP78, CHOP) were evaluated on U251, U-87, and A172. Lanes (1) vehicle, (2) POH 0.5 mM, (3) POH 1.5 mM

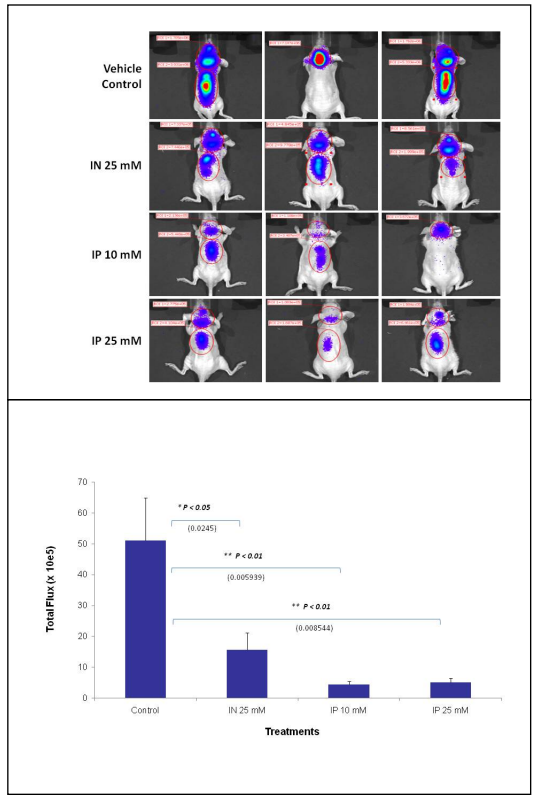

Fig. 5 Imaging demonstrates that tumor size is decreased after intranasal delivery of POH (A). Tumor-bearing animals treated with vehicle-DMSO, IN 25 mM POH, IP10 mM, and Intraperitoneally (IP) 25 mM POH, and monitored on a weekly basis. (B) IN treatment significantly reduced tumor growth.

Conclusions

Our data demonstrated POH is an effective anti-glioma agent for recurrent TMZ resistant gliomas which can be administered intranasally. A purified GMP quality POH has been prepared, and is in the process of obtaining IND approval for a Phase I/IIa clinical trial for GBM

Learning Objectives

Concept of intranasal delivery for gliomas; concept of endoplasmic reticulum stress