PAM-OBG: A MAOB SENSITIVE PRODRUG INHIBITOR OF MGMT POTENTIATES CHEMORADIOOTHERAPY

Martyn A. Sharpe, Sudhir Raghavan & David S. Baskin

Kenneth R. Peak Brain & Pituitary Tumor Center
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Introduction

Design and function of PAM-OBG: A bifunctional pro-drug for treating Glioma:

MGMT is capable of detoxifying TMZ generated O\textsuperscript{6}-methylguanine–DNA adducts and high levels of active MGMT in GBM are the greatest barrier to favorable clinical outcome in patients.

MAOB is highly expressed in glioma, with its expression directly correlated with tumor grade and inversely proportional to patient outcome. MAOB has the ability to oxidize propylamine-ethers. We used this reactivity to conjoin the MGMT inhibitor O\textsuperscript{6}-benzylguanaine (O\textsuperscript{6}-BG) to propylamine, via a carbamate, to generate a MAOB-specific anti-gliomal, prodrug.

Upon reaction with MAOB our prodrug immolates generating O\textsuperscript{6}-BG, thus inhibiting MGMT.

In addition, the acrolein generated reacts with DNA and gives rise to both intra- and inter-strand DNA crosslinks. As we have identified a weakness in the DNA-repair pathways that typically remove these lesions we hoped that our bifunctional prodrug would be capable of potentiating standard-care, Stupp-chemoradiotherapy.

We demonstrate that PAM-OBG is only activated by MAOB, it matures to O\textsuperscript{6}-BG deactivating MGMT, and that it is highly effective in the treatment of primary human glioma, \textit{in vitro} and in \textit{in vivo} intracranial mouse models.
Human glioblastoma multiforme primary cultured cells, GBM157, have been maintained in mouse flank since harvesting and used in vitro and in vivo experiments. PAM-OBG was synthesized from O\textsuperscript{6}-BG by first protecting the N\textsuperscript{9} nitrogen and then attaching trifluoroacetamide-protected aminopropanol to the carbamate immolative linker (Left).

We developed a ‘clickable’ MGMT probe (O\textsuperscript{6}PGG) which transfers a propargyl moiety to MGMT. The MGMT-ethyne adduct was visualized by FITC-PEG\textsubscript{5000}-azide/Cu/Asc.

The procedure for measuring the time-course of MGMT inactivation is shown in cartoon form left.
Results 1: PAM-OBG is a MAOB substrate and potentiates TMZ toxicity

In Figure 1, we show the enzymology of PAM-OBG with respect to hrMAOB and hrMAOA. (Left). PAM-OBG is a good MAOB substrate with a Km of 200 µM and a Vmax of about 18% of its favored substrate, benzylamine. At saturating levels of PAM-OBG, 750 µM, MAOA has <10% the activity of MAOB. The panel on the right shows a TMZ titration of primary, MGMT-positive, primary gliomal cultured cells. In these cells the IC₅₀ for TMZ is ≈800 µM. If the systemic inhibitor of MGMT, O⁶-BG, is pre-incubated the cells are sensitized to TMZ with the IC₅₀ falling to 290 µM. Pre-incubation with PAM-OBG is more effective than O⁶-BG as sensitization, giving an IC₅₀ of ≈90 µM. PAM-OBG activity is blocked with the MAOB inhibitor Selegilene.
Results 2: PAM-OBG is a MAOB substrate that inactivates MGMT

Figure 2. PAM-OBG acylates MGMT via MAOB

In Figure 4, we demonstrate that MAOB is able to bioconvert PAM-OBG into O\textsuperscript{6}-BG and this activity is abolished by the MAOB inhibitor Selegiline. Using our new MGMT assay we visualized active MGMT levels in cells incubated with O\textsuperscript{6}-BG (10μM), PAM-OBG (100μM)±Seg or vehicle for one hour and then added a ‘clickable’ MGMT substrate and fixed cells. The ‘clicked’-MGMT was visualized with FITC-azide. It can be seen that O\textsuperscript{6}-BG abolishes active MGMT, and that there is an 80% reduction in the levels of active MGMT with incubation with PAM-OBG. The control cells pre-treated with a MAOB inhibitor are as bright as the DMSO control cells, indicating that there has been no PAM-OBG to O\textsuperscript{6}-BG conversion. The panel on the right shows the time course of MGMT deactivation by PAM-OBG in gliomal cells. The degree of MGMT activity is both [PAM-OBG] and time dependent and loss of MGMT is at least as good as that observed with O\textsuperscript{6}-BG.
Results 3: PAM-OBG potentiates BCNU/CCNU in intracranial GBM

Figure 3. PAM-OBG sensitizes intracranial primary MGMT-positive GBM to alkylation in mouse intracranial models

In Figure 3 we show that PAM-OBG can potentially be used in BCNU/CCNU salvage therapy. Nude mice were given intracranial primary human GBM xenografts and were treated on days 10, 12 and 14. Treatment was i.v. BCNU or CCNU, given 1 hour after PAM-OBG or vehicle, or two vehicle injections.
Results 4: PAM-OBG potentiates Stupp protocol in intracranial GBM

Figure 4. PAM-OBG sensitizes intracranial primary MGMT-positive GBM to mini-Stupp chemoradiation

In Figure 3 we show that PAM-OBG can potentially be used in Stupp therapy. Nude mice were given intracranial primary human glioblastoma xenografts and were treated on days 13, 15, 17 and then on days 20, 22 and 24. Treatment was *i.v.* PAM-OBG or vehicle, 2 hours later mice were given TMZ or gavage vehicle, and then an hour later the anesthetized animal’s bodies were shielded and had either real or sham radiation to a dose of 2Gy.

Statistical significance between the PAM-OBG/TMZ/2Gy treatment group and the TMZ/2Gy treatment reached a *p*-value of <0.05 on day 22 and fell to <0.01 on day 27.
We looked for enzymes that were highly upregulated in glioma which could be used to catalyze the transformation of prodrugs into active chemotherapeutics.

We identified monoamine oxidase B as such an enzyme and designed a prodrug substrate for this enzyme.

PAM-OBG is a prodrug, inert, version of the MGMT inhibitor O\textsuperscript{6}-BG.

Oxidation of PAM-OBG by MAOB generates hydrogen peroxide, O\textsuperscript{6}-BG and acrolein.

In GBM cells both \textit{in vitro} and \textit{in vivo} the oxidation of PAM-OBG sensitizes cells to DNA alkylating agents due to the ablation of MGMT, via generated O\textsuperscript{6}-BG.

The acrolein generated by MAOB’s action on PAM-OBG is also a chemotherapeutic, causing the formation of DNA-adducts, leading to DNA breaks and interstrand DNA-crosslinks.

PAM-OBG was designed from the outset to be used in conjunction with the Stupp protocol and shows high efficacy in intracranial GBM mouse models treated with this regime.
Summary

1) We have previously shown that monoamine oxidase B is highly elevated in GBM and in low grade glioma.

2) MGMT is the main means that GBM use to evade TMZ-chemotherapy.

3) We have designed, synthesized and tested a MAOB sensitive prodrug, PAM-OBG, that generates both O\textsuperscript{6}-BG and acrolein only via MAOB catalytic activity.

4) The O\textsuperscript{6}-BG generated when glioma are treated with PAM-OBG can abolish MGMT activity and sensitize them to alkylating agents, \textit{in vitro} and \textit{in vivo}.

5) The acrolein generated when glioma are treated with PAM-OBG is a DNA-reactive chemotherapeutic in its own right.

6) PAM-OBG administration provides a six-fold improvement in lifespan in nude mice, with human primary GBM xenografts, when treated with TMZ/Radiotherapy (Stupp Protocol) or BCNU/CCNU salvage therapy.