Exploring alternative anti-glioblastoma candidate

[1]Patricia E. Mensah, [2]Annamarie Macaluso, [2]Diana Polania Villanueva, [2]Krzysztof Reiss
[1]LSU Health Sciences Center School of Medicine, New Orleans, LA
[2]Department of Interdisciplinary Oncology, LSU Health Sciences Center, New Orleans LA

Rationale

- The main obstacle for developing new and more effective glioblastoma therapies is inability of anticancer drugs to penetrate the Blood Brain Barrier (BBB).
- Our new anti-glioblastoma therapeutic approach is based on previously reported anti-glioblastoma activity of a common lipid-lowering drug fenofibrate (FF) that kills glioblastoma cells by a direct interaction with mitochondrial membranes resulting in a severe inhibition of the mitochondrial respiration.
- However, FF does not cross the BBB, and its cytotoxicity is attenuated by the high glucose content.

Aims

- Compare the chemical structure, physicochemical parameters, cytotoxicity, and metabolic effect of FF derivative, PP21.
  - Measure the cytotoxicity of PP21 in glioblastoma cell line and human derived glioblastoma spheroids.
  - Measure the metabolic effects of PP21.
  - Measure the concentration of PP21 accumulated in tissues after administration via oral gavage.

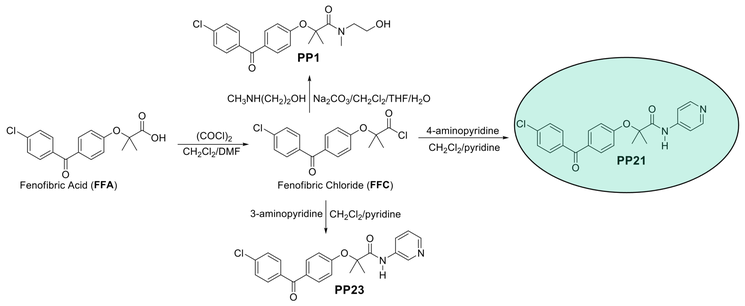
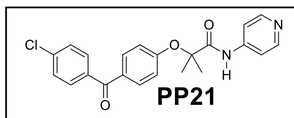


Figure 1. PP21 is a chemically modified derivative of Fenofibrate. It has improved anticancer efficacy and more effective brain tissue accumulation.

PP21 physicochemical parameters predict its increased ability to penetrate the BBB



Calc.	MarvinSketch 24.1.2		IP	RB	HLB	logS	PL	MPA	MSA	HBA	MR	$hERG_{AM}$	$hERG_{CM}$
		T0	8.56	6	10.46	-5.89	41.78	44.70	551.04	7	109.31	5.28	SAFE
ClogP	4.58	0.21											
ClogD	4.57	0.00											
MW	394.86	0.75											
PSA	68.29	1.00											
HBD	1.00	0.75											
pKa	4.85	1.00											
CNS-MPO	3.71												
BBB_Score	4.50												

Figure 2. IP = Isoelectronic Point; RB = Number of rotatable bonds; HLB = hydrophilic-lipophilic balance; logS = water solubility at pH 7.4; PL = polarizability(Å^3); Minimal Projection Area (Å^2); MSA = molecular surface area; HBA = hydrogen bond acceptor sites; HBD = hydrogen bond donor sites; MR = Molar refractivity; hERG = estimated pIC50 (pAct) value for hERG (the human ether-a-go-go (hERG) ion channel); $hERG_{AM}$ = hERG activity model; $hERG_{CM}$ = hERG classification model; ClogP = partition coefficient; ClogD = distribution at pH = 7.4; PSA = polar surface area; HBD = hydrogen bond donors; pKa = estimated acid strength; CNS-MPO = 3.71 score for CNS penetration; BBB_score = 4.50 score for blood-brain barrier penetration.

Penetration of PP21 through model membrane of BBB

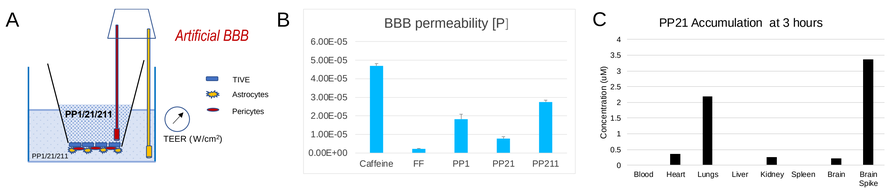


Figure 3. *Penetration of PP1, **PP21** and PP211 through in vitro BBB model membranes:* ***Panel A:*** *Schematic representation of a triple-coculture model of the BBB, which consists of astrocytes, pericytes and epithelial cells cultured on 24-well transwell membranes with 3μm pores. Trans-endothelial electric resistance (TEER) was measured to confirm proper formation of the BBB, using a EVOM^2 meter with a STX3 electrode (World Precision Instruments).* ***Panel B:*** *BBB permeability (P) for PP1 (experimental drug) and PP211 (prototype drug) was calculated using $P=V_A \cdot C_A/(t \cdot S \cdot C_L)$ equation (39) and normalized by the corresponding TEER values. FF was used as negative control and caffeine as positive control for the BBB penetration. Data represent average values from 2 independent experiments in duplicate (n=4) with standard deviation SD. * indicates values significantly different from FF, ** indicates values significantly different from PP1.* ***Panel C.*** *PP21* accumulated in tissues after administration via oral gavage after 3 hours.

Effects of PP21 on intracellular ATP and glucose content in the medium

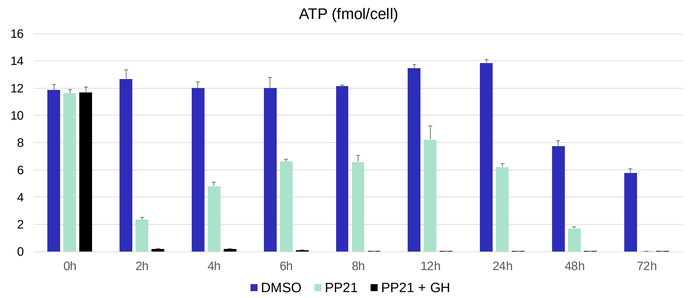
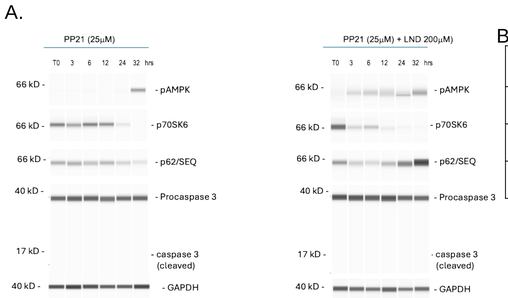


Figure 4. *Effects of PP21 on intracellular ATP and Glucose content in the medium. ATP level was measured in low glucose (1g/L) using CellTiter-Glo 2.0 (Promega) and glucose content by Gluco-Glo (Promega) at indicated time points following DMSO (control) PP1, PP1+LND, and PP1+GH treatments. Data represent average values with standard deviation (n=3). *Indicate values significantly different from 1h time point ($P \leq 0.05$). #GH – gnetin H (a natural product found in Vitis vinifera and potent inhibitor of glycolysis); ##LND – Lonidamine (BBB -penetrable inhibitor of glycolysis). Values below the histogram show % LN229 cell death evaluated by trypan blue exclusion test. The cells were cultured in DMEM low glucose medium (1g/L) + 10% FBS.*

PP21 induces necrotic cell death, drop in ATP, activation of AMPK-induced autophagy

A.



B.

	PP21	Control (vehicle)
24 hours	4.5%	1.6%
48 hours	93.4%	3.2%
72 hours	99.1%	3.2%

Figure 5. A. *Capillary Western analysis showing a severe metabolic disturbance triggered by PP21. Total proteins were extracted at indicated time points for the detection of: pAMPK (Thr172) (low-energy sensor); p70S6K (Tyr389) (marker of active translation); SQSTM1/p62 (marker of active autophagy); Caspase 3 antibody that recognizes both pro-Caspase 3 and cleaved-Caspase 3 (marker of apoptosis), and loading control, GAPDH.* B. necrosis-like cell death induced by PP21 demonstrated by survival study and Trypan blue staining. Vehicle = DMSO

Comparing cytotoxicity of PP21 to FF

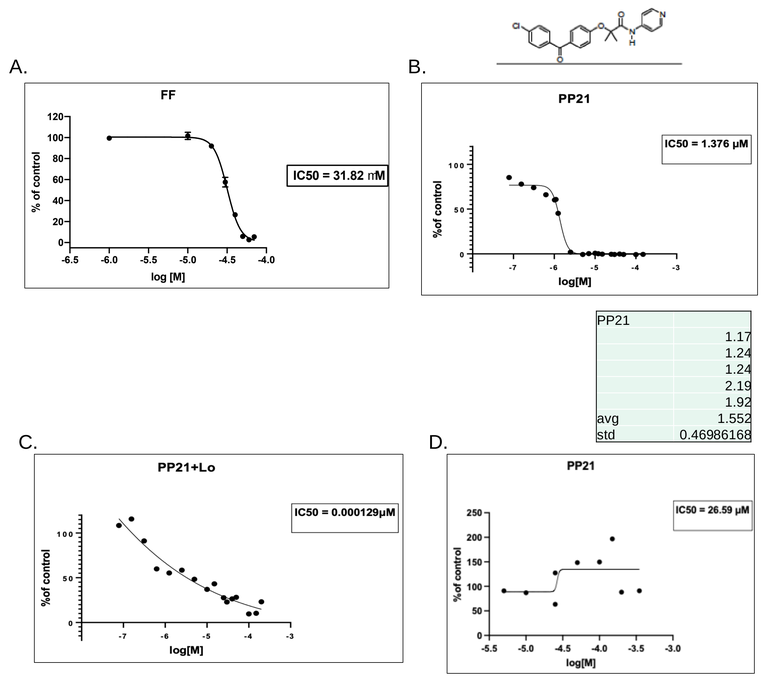


Figure 6. The toxicity of PP21 was tested in a variety of circumstances to compare to that of Fenofibrate. **A.** Fenofibrate has an IC50 of 31.81. **B.** Glioblastoma IC50 of PP21 tested in LN229 was over 20-fold lower than Fenofibrate. Average IC50 of 6 additional experiments determined to be 1.552 uM. **C.** Results of glioblastoma IC50 tested in LN229 with glycolysis inhibitor, Lonidamine, are inclusive as more concentrations will need to be tested to get a true IC50. **D.** PP21 IC50 tested in human-derived GBM12 sphere cultures are inclusive and ongoing work aims to deduce the best technique to measure it.

Conclusion

- The observed cytotoxicity involved a severe and immediate blockade of mitochondrial respiration followed by increased glycolysis (not shown), which in low glucose environment (1g/L), triggered a severe drop of intracellular ATP, activation of AMPK-induced autophagy, and ultimately necrosis-like glioblastoma cell death.
- Addition of glucose attenuated PP21-induced glioblastoma cytotoxicity. Therefore, we tested a new approach to challenge glucose-dependence of the PP21 treatment, which involves addition of specific glycolysis inhibitors: lonidamine (LND).
- Our cell culture data show that PP21+LND, is cytotoxic to glioblastoma cells and helped eliminating glioblastoma cells in a high glucose environment.
- Our data show that PP21 can penetrated both artificial BBB model membranes and, importantly we have detected PP21 in the brain tissue at clinically relevant concentrations following oral gavage drug delivery.