Using tandem mass spectrometry to study the blood-brain-barrier permeability of a novel cyclic peptide in a rodent model

Kasuni Gamage[1], Mila Sajinovic[2], Kieran F. Scott[2,3], Shadma Fatima[2, 3] and David G. Harman[1]

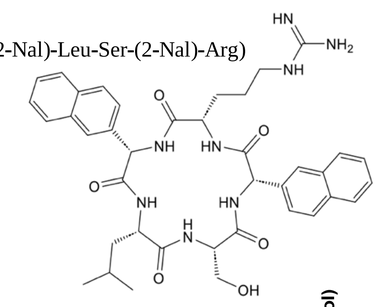
1. School of Science, Western Sydney University 2. Ingham Institute of Applied Medical Research 3 School of Medicine, Western Sydney University

19243506@student.westernsydney.edu.au

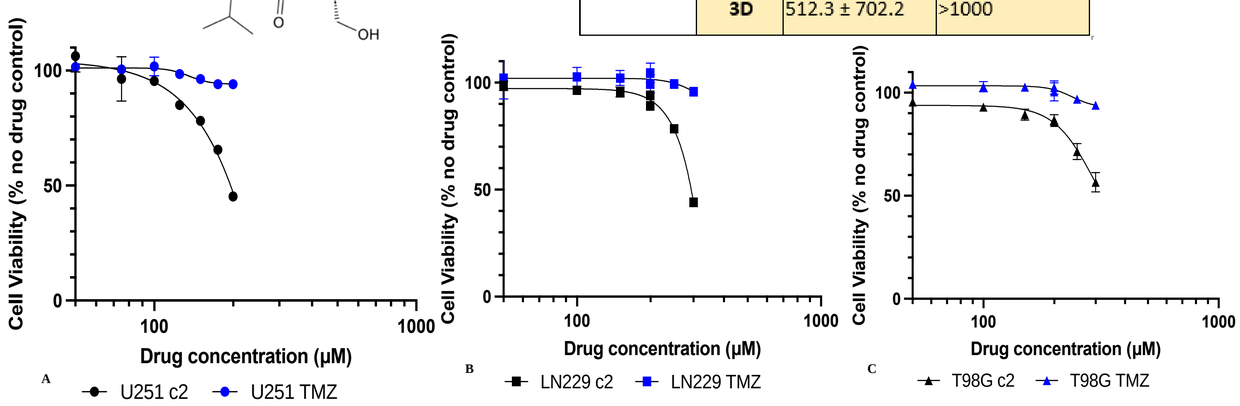
Background and Aim

- Glioblastoma multiforme (GBM) is the most common and aggressive form of primary brain tumour in adults, with an incidence rate averaging 6.9 cases per 100000 people annually.
- Despite an invasive multidisciplinary approach to treatment involving surgical resection, followed by radiotherapy and chemotherapy, most patients experience tumour recurrence resulting in median survival of fewer than 15 months after diagnosis.
- A key reason contributing to poor patient outcomes is resistance to the current standard care temozolomide (TMZ). Considering the development of drug resistance and high recurrence of GBM, there is an urgent need for new treatments.
- We have determined the ability of the new drug c2 to kill three GBM cell lines in 2D and 3D experiments.
- As there is a vast array of complexities surrounding the blood-brain barrier (BBB), it can be considered the bottleneck in brain drug development and one of the most crucial factors limiting the growth of glioma neurotherapeutics.
- This study was conducted with the aim of determining if c2 crosses the blood-brain barrier in a healthy rodent model.

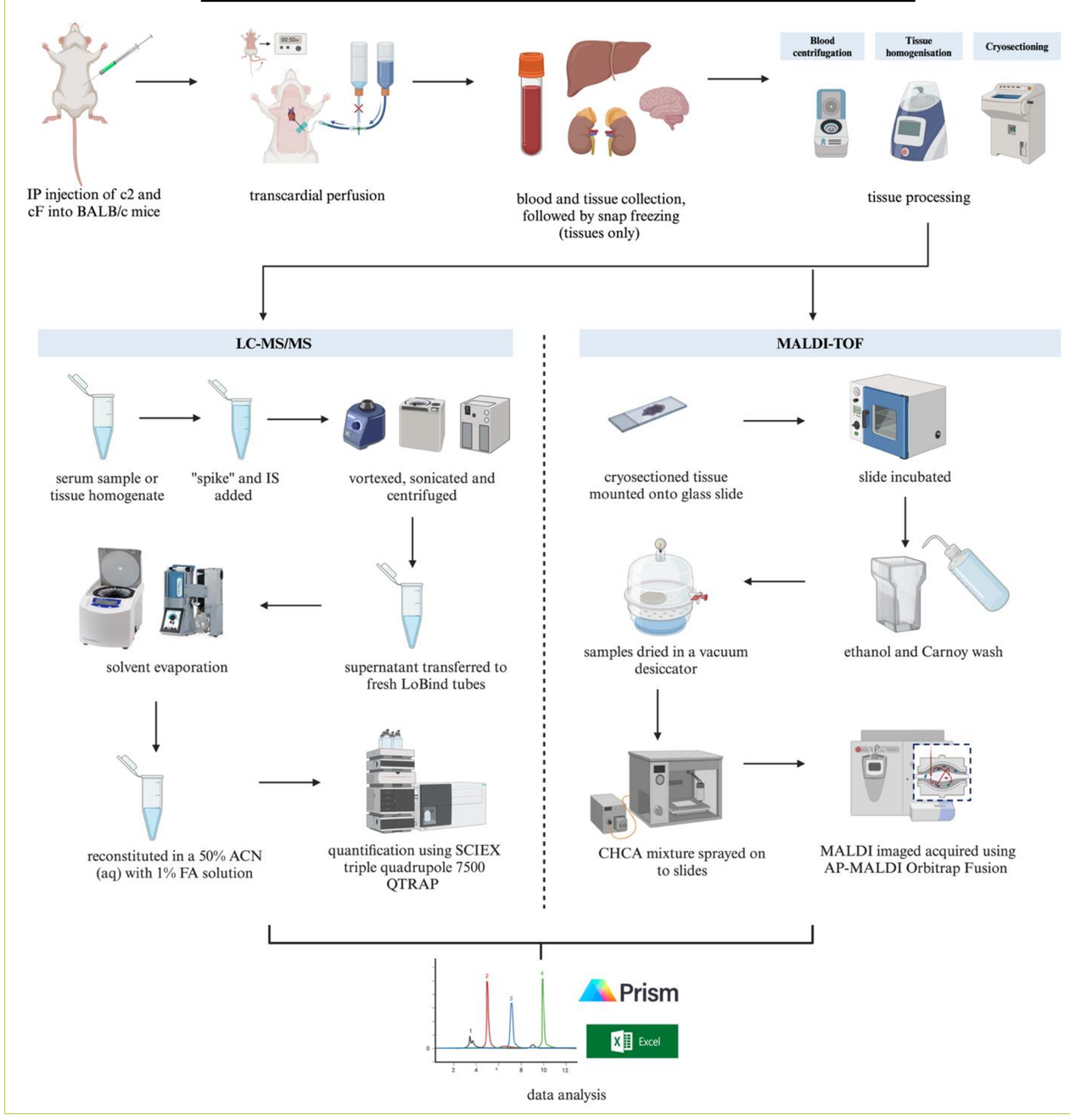
c2. cyclo-((2-Nal)-Leu-Ser-(2-Nal)-Arg)



		IC50 (μM)	
		c2	TMZ
U251	2D	168.3 ± 3.2	417.3 ± 5.5
	3D	184.6 ± 13.0	433.4 ± 37.5
LN229	2D	199.6 ± 5.5	430.2 ± 6.1
	3D	336.3 ± 83.0	720.1 ± 199.9
T98G	2D	330.8 ± 33.3	>1000
	3D	512.3 ± 702.2	>1000

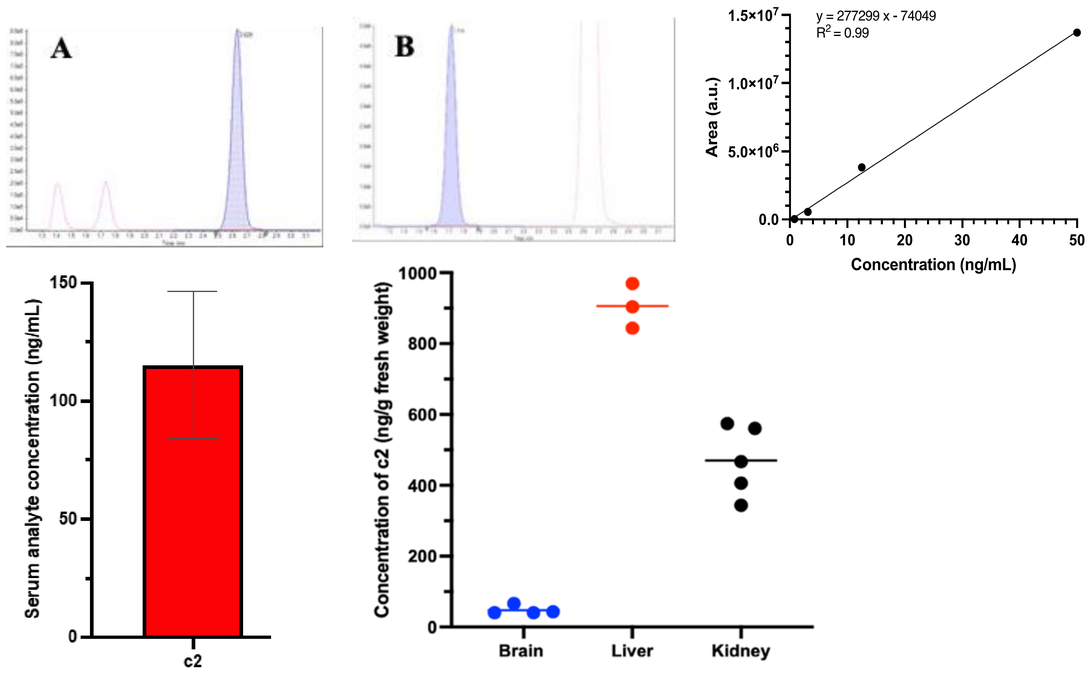


Materials and Methods



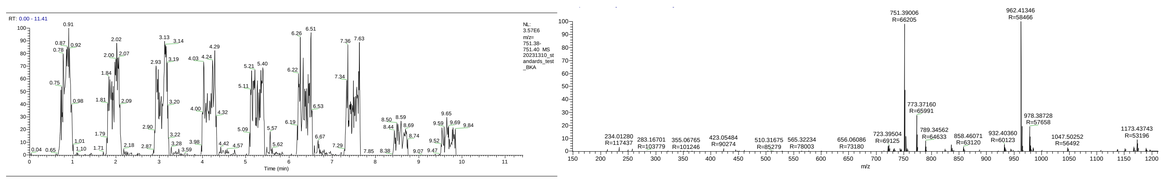
Results and Discussion

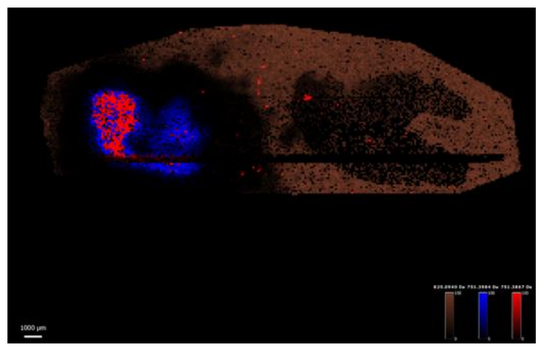
- In the **LC-MS/MS** analysis, a structural analogue of c2, cF, was used as the internal standard. These analytes were previously quantified in the lab.
- The retention time (RT) of c2 was observed at 2.62 ± 0.011 min and cF was observed at 1.72 ± 0.013 min.
- The unknown concentration of analyte in serum was calculated using a standard curve. These results indicated that the sample preparation and analysis methods allow c2 and cF to be stable enough to be quantified reliably., i.e. serum. c2 concentration in mouse serum was calculated as 115 ± 66 ng/mL (n=5).



	Serum (ng/mL)	Liver (ng/g fresh weight)	Kidney (ng/g fresh weight)	Brain (ng/g fresh weight)
c2	115 ± 66	995 ± 46	322 ± 132	50 ± 14

- In the **MALDI** analysis, an off-tissue peptide standard test for 5.3 mM c2 discovered that c2 peptide m/z 751.39006 seems co-localise with [c2+M-H+Na]+ adduct m/z 962.41346.





- Red channel shows m/z 751.3867, which is the suspected exogenous peptide. But possible isobaric overlap with blue channel m/z 751.3984 with a distinct spatial distribution was seen. Both m/z signals were depleted by washing. Potentially, there is no need to wash with AP-MALDI.
- Signal from brain tissue sections subjected to the ethanol-Carnoy wash and those unwashed were compared. These results indicate the presence of c2 peptide ion peak m/z 751.3867 in various regions of the brain. However, drawing definitive conclusions is challenging due to the potential co-localization of this peak with the [c2+M-H+Na]+ adduct at m/z 962.4015. Additionally, there may be isobaric overlap with the peak at m/z 751.3984, though this peak displays a distinct spatial localization within the brain.

Conclusions and Future Work

- LC-MS/MS confirmed the detection of c2 in healthy mice brains.
- While the MALDI-MSI results were only preliminary, they clearly indicate the presence of c2 peptide ion peak *m/z* 751.3867 in various regions of the brain. Further optimisation is required to ascertain that this specifically corresponds to c2.
- These results, confirm that c2 crosses the murine BBB and we predict that achieving therapeutic concentrations in human patients is feasible.
- Next, we will be inoculating mice at a lower dose to determine if c2 can still be detected, followed by repeating the experiment on a GBM model to see if the results are reproducible across different models.