Effect of topoisomerase inhibitors in glioblastoma animal models: A meta-analysis and systematic review

Toni Rose J. Jue¹, Theodore Hirst², Emily Sena² and Kerrie L. McDonald¹

¹ Cure Brain Cancer Neuro-oncology Group, Prince of Wales Clinical School, UNSW Australia; ²Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

Introduction
Glioblastoma multiforme (GBM) is the most common malignant tumour occurring in adults, with a median survival rate of 15 months from the time of diagnosis. The current standard of treatment is bulk tumour removal followed by radiotherapy with concomitant and adjuvant temozolomide (TMZ) chemotherapy. The prognosis to TMZ response can be determined by analysing the methylation status of methylguanine-DNA methyltransferase (MGMT). A methylated MGMT can be found in approximately 25-40% of the GBM population. Patients with methylated MGMT respond better to TMZ chemotherapy, thus increasing the 2 year survival to 46% (Hegi et al., 2005). Numerous preclinical and clinical studies are being conducted to provide treatment to the other 60-75% of the population which has unmethylated MGMT. Other approved treatments for GBM include bevacizumab and nitrosoureas (CCNU/BCNU wafers). However, even with the availability of these approved therapies a significant change in the survival of GBM patients has yet to be observed. Novel therapies are still being investigated to find a possible treatment for those unresponsive patients.

One of the novel drugs being investigated for the treatment of GBM are topoisomerase inhibitors. Topoisomerase inhibitors target topoisomerase 1 and 2, both enzymes are responsible in catalysing DNA single or double stranded breaks. Topoisomerase inhibitors cause cell death by forming stable topoisomerase-cleaved DNA complexes (Ataka et al., 2007; Burden & Osheroff, 1998). The aim of this meta-analysis is to get a wider perspective of the effects of topoisomerase inhibitors in the treatment of GBM in animal tumour models, with this data we can compare its efficacy to the results that we see in clinical studies.

Research question
What is the effect of topoisomerase inhibitors on tumour volume and survival in glioblastoma animal models?

Objective
1. To gather published articles relevant to the use of topoisomerase inhibitors in the treatment of GBM models in different databases.
2. To extract data from these published articles.
3. To perform a statistical analysis on these extracted data to evaluate the efficacy of topoisomerase inhibitors on glioma animal models by analysing survivability and changes in tumour volume.
4. To formulate a sound and comprehensive conclusion based on these statistical results.
5. To present the results in the form of a meta-analysis and a systematic review, as reference to future pre-clinical and clinical research on the use of topoisomerase inhibitors as treatment for GBM.

Methods
Literature search
A literature search of FDA-approved topoisomerase inhibitors that are most commonly used for GBM phase II clinical trials will be performed. Public databases that will be used for the literature search include Embase and Pubmed. Keywords that will be used for the search include, (glioblastoma or
glioblastoma multiforme or GBM or high grade glioma) AND (Doxorubicin OR Epirubicin OR Etoposide OR Irinotecan OR Topotecan). Search will be limited to animals according to predeveloped filters (de Vries, Hooijmans, Tillema, Leenaars, & Ritskes-Hoitinga, 2011; Hooijmans, Tillema, Leenaars, & Ritskes-Hoitinga, 2010). No language restrictions will be applied.

**Selection criteria**

Selection criteria for inclusion in the data analysis:

<table>
<thead>
<tr>
<th>Intervention</th>
<th>FDA-approved topoisomerase inhibitors evaluated as monotherapy (Food &amp; Administration, 2010)</th>
</tr>
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</table>
|              | • Doxorubicin
|              | • Epirubicin
|              | • Etoposide
|              | • Irinotecan
|              | • Topotecan
| Population   | Adult high-grade glioma animal models
| Treatment Groups | Comparison of individual drug groups against control group must be available
| Tumour implantation | Intracranial or subcutaneous
| Outcome measures | Tumour volume, survival

The reference list of selected articles will also be screened for additional papers. The articles will be initially screened based on the title and, consequently, the abstract. Final inclusion will be based on the screening of the full text of remaining articles. A report on the screening process will be reported based on the PRISMA flow chart (Figure 1)
Figure 1. Adapted from PRISMA’s preferred reporting system on the flow of information in the different phases of a systematic review. (Moher, Liberati, Tetzlaff, & Altman, 2009)

Assessing methodological quality and publication bias
A modified 11-point checklist (Amarasingh, Macleod, & Whittle, 2009; Hirst et al., 2013) will be used to assess the quality and determine publication bias. If approved, the data will be entered into the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) data manager application in Microsoft Access 2003.

### 11-point checklist for assessing methodological quality and publication bias

1. Peer-reviewed publication
2. Sample size calculated
3. Randomised allocation of drug (or control) treatment
4. Blinded assessment of outcome
5. Compliance with animal welfare regulations
6. Statement of conflict of interests
7. Uniform number of cells implanted
8. Site of implantation is consistent in all animals
9. “Take rates” of implanted tumour cells is mentioned in the publication
10. Number of excluded animals must be stated with reasons for exclusion mentioned
11. Drug delivery method mentioned with the justification of why this particular method was used (i.e., convection enhanced delivery, nanoparticles, etc)

Data Extraction
Data to be extracted will include the following:

| Study Identification | 1. Author  
|----------------------|-----------|
|                      | 2. Year of Publication  
|                      | 3. Title  

| Intervention | 1. Topoisomerase inhibitors must include:  
|--------------|-------------------------------------------|
|              | a. Doxorubicin  
|              | b. Epirubicin  
|              | c. Etoposide  
|              | d. Irinotecan  
|              | e. Topotecan  
|              | 2. Dosage  
|              | 3. Frequency of administration  
|              | 4. Route of administration  
|              | 5. Drug delivery method (i.e., convection enhanced delivery, nanoparticles, etc)  
|              | 6. Delay to treatment (number of days from day 0/starting point)  

| Animal population | 1. Species  
|-------------------|-----------|
|                   | 2. Strain  
|                   | 3. Sex  
|                   | 4. Age  
|                   | 5. Glioma cell type used  
|                   | 6. Population size defined  
|                   | 7. Number of animals in each group  

| Tumour implantation | 1. Site of implantation  
|--------------------|--------------------------|
|                    | a. Intracranial  
|                    | b. Subcutaneous/Flank  
|                    | 2. Number of cells inoculated  
|                    | 3. Inoculation method  

| Outcome measures | 1. Published numerical values for the following measures of  
|------------------|--------------------------|


Outcomes:
- Change in tumour volume
- Median survival

2. Method of tumour volume measurement

Data Analysis
Gathered data will be statistically analysed and compared between treatment and control groups, pooled using DerSimonian and Laird random-effects meta-analysis; this will also assess for the presence of between-study heterogeneity.

If present, we will attempt to partition between-study heterogeneity using stratified meta-analysis. We will search for evidence of publication bias using funnel plots, Egger regression and ‘Trim and Fill’ analysis (Duval & Tweedie, 2000).

<table>
<thead>
<tr>
<th>Data</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour volume</td>
<td>Log transformed tumour volume ratio</td>
</tr>
<tr>
<td>Median survival</td>
<td>Median survival ratio</td>
</tr>
<tr>
<td>Pooled Data</td>
<td>Random effects model</td>
</tr>
</tbody>
</table>

References


