

What is the translational efficacy of chemotherapeutic drug research in neuro-oncology? A systematic review and meta-analysis of the efficacy of BCNU and CCNU in animal models of glioma

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Abstract *Introduction* The translational value of experimental therapeutic neuroscience research to clinical practice is highly variable. This has been particularly well demonstrated in the field of neuroprotective agents following either head injury or stroke. In this study we evaluate the efficacy of systemic BCNU and CCNU in experimental glioma models and how the experimental data has translated into clinical practice. *Methods* A systematic review of the efficacy of BCNU and CCNU, against experimental rodent and murine in vivo glioma models was conducted. Selected articles were graded on a 15 point scale for scientific methodology. A stratified meta-analysis based on median-survival data and effect sizes was performed to generate global-efficacy estimates for BCNU and CCNU, and to produce ‘weighted-mean effect-sizes’ for individual sub-categories of selected study-characteristics. *Results* Fourteen papers satisfied search criteria and encompassed 231 treatment comparisons in 2256 animals. The median methodology score was 9 (range 7–12/15). Global-efficacy estimates were BCNU 0.194 (95% CI –0.538 to 0.927) and CCNU 0.432 (95% CI –0.392 to 1.256), with CCNU being significantly more effective than BCNU. Because of these wide confidence intervals a beneficial or detrimental effect of either agent could not be

confirmed. Most selected study-design characteristics (e.g. glioma cell line, drug dosage, drug scheduling, mode of drug administration, timing of therapy after glioma implantation but not animal used) significantly influenced the efficacy-results obtained. The methodological score did not influence efficacy-estimate. *Conclusion* This review has found (i) experimental-design influenced the efficacy-data obtained and (ii) that there is highly variable outcome data for the efficacy of both BCNU and CCNU in experimental in vivo rodent and murine glioma models. In many ways these findings are analogous to the use of nitrosoureas in human malignant glioma. The statistically significant small beneficial effect of nitrosoureas in combination with other chemotherapeutic agents in human glioma was only noted after a meta-analysis of human randomized controlled trials.

Keywords CCNU · BCNU · Experimental glioma model · Glioma chemotherapy

Introduction

The nitrosoureas carmustine (BCNU) and lomustine (CCNU) are chemotherapeutic agents commonly used in the management of malignant gliomas. As alkylating agents their oncolytic effect is due to reactions between metabolic intermediates (diazoalkanes) with nucleic acids and proteins, resulting in covalent interlinking of DNA strands. This DNA damage impedes further cell division and targets cells for degradation by apoptosis [1–3]. Because of their lipophilic nature both BCNU and CCNU readily penetrate the blood brain barrier. Both BCNU and CCNU accumulate in brain, with tissue/plasma ratios of up to 4:1 [4].

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Studies of the efficacy of BCNU and CCNU in rat and mouse models of glioma were first reported in the 1960s. These and later studies suggested that both agents were effective *in vivo* in a wide range of brain tumour models and that nitrosourea treatment significantly extended median survival and reduced tumour volume [5–27]. Nitrosoureas were subsequently introduced into clinical practice in the 1960s [28, 29]. A recent systematic review [30] and a meta-analysis of human clinical trial data [31] suggests that BCNU and CCNU, often in combination with other agents, have a small, but statistically significant effect on survival of patients with malignant glioma. The response to BCNU and CCNU in both experimental glioma and in human clinical trials is limited largely due to molecular chemoresistance, mediated principally by the multidrug resistance genes *O*⁶-methylguanine DNA methyltransferase (MGMT) and glutathione S-transferase [32].

This contrast between substantial reported efficacy in some animal studies and at best modest efficacy in well conducted human clinical trials has also been observed for other conditions including stroke and head injury [33]. Subsequent exploration of the potential causes for this discordance suggest firstly that the animal literature may be substantially biased by the confounding effects of certain aspects of study design (including randomisation, allocation concealment and blinded outcome assessment) [34–36] secondly that clinical trials may have tested efficacy under circumstances in which efficacy was not seen in animals [33, 35] and thirdly that clinical trials might have been conducted before the characteristics of and limits to efficacy in animals was established [37, 38].

Modelling the responsiveness to chemotherapy is very different to modelling the response to cerebral ischaemia. While the immediate therapeutic target (the tumour cell) can be identical to that causing human disease (in contrast to stroke, where the pathophysiology may be different) the differences between tumour and host cells may be exaggerated in animal models. The differences between experimental *in vivo* models of glioma and the human disease have previously been highlighted [39, 40]. These

features could exaggerate the experimental efficacy and tolerability of chemotherapeutic agents. In neuro-oncology this has best been highlighted by the success of gene therapy paradigms in experimental glioma [41, 42], but their failure in a Phase III randomized controlled clinical trial [43–45]. In spite of these difficulties, testing efficacy in animal models of glioma remains a key stage in the development of novel therapeutic drugs.

Here, we report a systematic review and stratified meta-analysis of the efficacy of BCNU and CCNU in experimental rodent and murine models of glioma to determine (1) concordance between an unbiased assessment of efficacy in animals with that observed in meta-analyses of clinical trials; (2) the scientific rigour in study design and outcomes; and (3) the impact of factors such as inter alia drug dose, timing of treatment, species, and reported study quality

Methods

Search-strategy and selection criteria

We conducted an electronic search of Pubmed (1974 to May 2007), MEDLINE (1950 to May 2007) and EMBASE (1980 to May 2007) using the search-terms (<glioma> OR <glioblastoma> OR <ependymoma> OR <brain tumour>) AND (<BCNU> OR <CCNU> OR <carmustine> OR <lomustine>) (limit to animals). We included studies testing the efficacy of BCNU or CCNU in animal models of experimental glioma where outcome was reported as change in tumour volume or survival compared with that observed in a control group. We specifically excluded studies reporting the efficacy of BCNU or CCNU in combination with other therapeutic agents (Table 1).

Methodological quality and publication bias

To estimate methodological quality [45–47] we derived a 15-item quality checklist based in part on the CAMARADES

Table 1 Selection criteria for published studies to be included in the analysis

1.	BCNU (carmustine) or CCNU (lomustine) alone
2.	Experimental animal (rat/murine) model
3.	Glial tumour (glioma) model utilised
4.	Systemic administration of BCNU/CCNU (i.e. intraperitoneal (IP); IV; IM; PO; intra-carotid)
5.	Intracerebral (IC) implantation of tumour
6.	Published numerical data of median survival for both control and treated animals ^a
7.	Quoted numbers of treated animal models used ^b

^a Tumour volume data was excluded from this analysis, as tumour volume reductions were recorded at different times in different experimental studies, and could not be compared fairly

^b Essential for ‘weighted-mean effect-size’ calculations (see Table 3)

Table 2 Fifteen point study methodology quality assessment criteria

1.	Peer-reviewed publication
2.	Sample-size calculation performed
3.	Randomised allocation of treatment (and control) drugs
4.	Blinded assessment of outcome
5.	Compliance with animal welfare regulations (appropriate living and feeding conditions)
6.	Statement of potential conflicts of interest
7.	Standardised number of cells extracted for implantation/standard tumour weight implanted
8.	“Take-rates” of implanted experimental tumours mentioned in paper
9.	Excluded animals mentioned in Results/discussion section, and reasons quoted, including anomalies
10.	Appropriate statistical analysis performed (e.g. Wilcoxon, Mann and Whitney U-test; $P < 0.05$)
11.	Justification of the brain-tumour used to study the efficacy of a chemotherapeutic agent (e.g. close histological resemblance to human brain-tumour ‘X’; reproducible model)
12.	Justification that chemotherapeutic agent is acting directly against brain tumour, and not on normal brain parenchyma (e.g. injecting drug into non-tumour bearing animals; in vitro-culture studies on normal brain tissue and/or glioma)
13.	Tumour-volume doubling-time/growth kinetics considered, or narrow range of median survival times for control (non-treated tumour-implanted) animals stated
14.	Consistent site of intracerebral (IC) implantation
15.	Drug solubilising media considered

study quality checklist [35] and in part on consideration of particular challenges in in vivo glioma (Table 2). Such checklists can only capture reported study quality, and an unweighted quality score was obtained by attributing 1 point for each quality item scored. The presence of publication bias was tested using a Funnel Plot of effect size against number of animals included.

Statistical analysis

From each publication we extracted data for individual comparisons, defined as the outcome for a group of animals treated with a specific dose of drug at a specific time in a specific glioma model compared with that in a control group.

To allow the survival outcomes from individual comparisons to be combined to give a global estimate of efficacy we defined the effect size as the difference between the reciprocals of survival in each group expressed as a proportion of the reciprocal of survival in the group

with the shortest survival (see Table 3). Where more than half the treated animal were alive at the latest time-point reported (i.e. median survival was at least as long as this latest time point) survival was considered, for the purposes of this analysis, to be infinite. In these circumstances the effect size would be 1.0; a doubling of survival in the treated group gives an effect size of 0.5; where survival was equal in both groups the effect size would be 0; and where survival in the treatment group was half that in the control group the effect size would be -0.5.

The basis of combining data using meta-analysis is that individual studies give imprecise estimates of the same phenomenon. That imprecision comes from biological variability in the population studies and from measurement error. In clinical trials measurement error is small and meta-analysis weights the outcome of individual studies according to the number of patients included in each study, as reflected in the inverse of the variance of the outcomes reported (larger studies having smaller variance). In spite of their much smaller size, meta-analyses of animal studies

Table 3 Interpretation of effect size in experimental studies of the efficacy of either CCNU or BCNU against experimental rodent or murine glioma

The larger the effect size (>0), the longer the treated animal lives, relative to controls

Effect-size score	Interpretation
$S^e < 0$	Treated animals survive for shorter period than control animals (i.e. toxic dose)
$S^e = 0$	No increase/decrease in median survival of treated, compared to control animals (i.e. equal median survivals in treated and control animals)
$0 < S^e < 1$	Median survival of treated animals greater than control animals
$S^e = 1$	Infinite survival of treated animal, compared to control animal (NB. S^e cannot be greater than 1)

have also conventionally weighted outcome using variance, on the basis that well conducted studies will be subject to less measurement error and therefore have lower variance.

In *in vivo* studies reporting median survival in animals with glioma the measurement error will be small and the best measure of median survival will be seen in the largest studies. To account for this we weighted our analysis according to the number of animals included, so that larger studies were given greater weight.

For both BCNU and CCNU a global estimate of efficacy was derived along with 95% confidence limits for that estimate. Stratified meta-analysis was carried out according to dose, treatment regimen, glioma model, species of animal, route of drug administration, and methodological score. The significance of differences between groups of studies was determined by analysis of variance. Where appropriate we used Tukey's HSD post hoc test to identify the source of differences between groups. We considered $P < 0.05$ to represent statistical significance.

Results

The electronic search identified 1,543 studies (BCNU 1,028; CCNU 515). 59 publications reported information potentially of interest, but 45 of these were excluded because there were insufficient data (16 publications); data on tumour volume only (8); the publications were unavailable (4), duplicate (3) or in a foreign language (1); the tumour studied was not glial (7); or for other reasons (6). This analysis is therefore based on data from 14 publications (5 BCNU; 7 CCNU; 2 combined) reporting data from 232 individual comparisons involving 2,256 experimental animals.

Methodological quality and publication bias

Overall, reported study quality was modest (median 9/15, range 7–12/15; IQR 8–10.5). There was no relationship between study quality and effect size (Fig. 1). The most frequently omitted methodological criteria were failures to report random allocation to control/treatment group, failure to report a sample size calculation and failure to report blinded assessment of outcome.

Efficacy

Treatment with either BCNU or CCNU had no significant effect on median survival (0.312, 95% confidence interval -0.502 to 1.126), and the wide confidence limits are equally consistent with a substantial beneficial or a detrimental effect. However, treatment with CCNU (0.432, 95% CI -0.392 to 1.256) was significantly more effective than

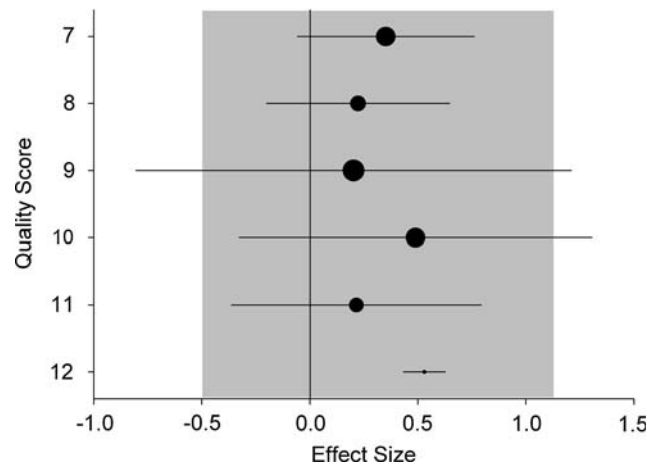


Fig. 1 Forest plot showing the relationship between methodological quality score of the paper (maximum 15) and effect size for the 14 reviewed papers. There was no statistical relationship between CCNU or BCNU efficacy and the quality of the paper

BCNU (0.194, 95% CI -0.538 to 0.927 ; $F_{(1,229)} = 22.3$, $P < 0.001$).

Total drug dose

For both BCNU and CCNU there was a clear dose–response relationship, with efficacy initially increasing as dose increased, and with higher doses of each drug associated with reduced efficacy (Fig. 2a). Doses of BCNU above 30 mg/kg were associated with reduced survival (-0.267 ; 95% CI -1.146 to 0.609 ; $F_{(3,117)} = 34.2$, $P < 0.001$). Doses of CCNU above 50 mg/kg were associated with reduced efficacy (0.264, 95% CI -0.939 to 1.468 ; $F_{(2,107)} = 5.14$, $P < 0.01$; Fig. 2b), increased mortality only being seen at doses higher than 70 mg/kg (-0.516 , 95% CI -0.776 to -0.256). Because of this potential for high (toxic) doses to cause harm and the likelihood that low doses would have limited efficacy we repeated the global analysis over what might be considered the therapeutic range for each drug (BCNU up to 30 mg/kg; CCNU up to 50 mg/kg), but even under these favourable conditions neither drug significantly improved outcome.

Timing and frequency of treatment

It is generally held that chemotherapy has the greatest prospect of success when initiated early. Surprisingly, for BCNU efficacy was higher with longer intervals from implantation with tumour cells to initiation of treatment [$F_{(3,117)} = 10.6$, $P < 0.01$; Fig. 3a]; in contrast, for CCNU efficacy was greatest when treatment was initiated within 2 days of inoculation, and fell thereafter [$F_{(3,106)} = 10.0$, $P < 0.01$; Fig. 3b].

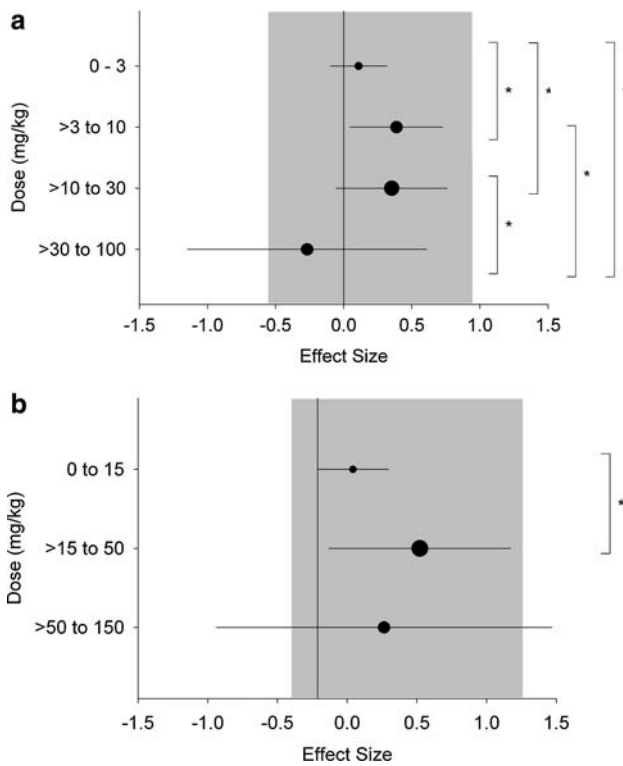


Fig. 2 (a) Forest plot showing dose–response efficacy for BCNU. Doses >30 mg/kg were associated with a significantly worse outcome. * Indicates $P < 0.05$. (b) Forest plot showing dose–response efficacy for CCNU. Doses >70 mg/kg were associated with a significantly worse outcome. * Indicates $P < 0.05$

There was also discordance between the BCNU and CCNU data regarding the relative efficacy of single versus multiple doses (Fig. 4). For BCNU there was no difference in efficacy [single dose 0.182 (95% CI –0.571 to 0.935) versus multiple doses 0.348 (95% CI 0.126–0.570), $F_{(1,119)} = 1.7$, ns: Fig. 3a], whereas for CCNU efficacy was significantly higher with single [0.513 (95% CI –0.331 to 1.358)] rather than multiple [0.177 (95% CI –0.307 to 0.660)] doses [$F_{(1,108)} = 11.5$, $P < 0.01$: Fig. 3b].

Glioma model

For BCNU, the method of induction of experimental glioma appeared to have a substantial impact on the efficacy seen ($F_{(9,111)} = 4.33$, $P < 0.001$); BCNU appeared to be more effective in cells of human origin, particularly the D45MG glioma cell line (0.530, 95% CI 0.433–0.627). There was also an improvement in survival with VDMk 497-P (0.281, 95% CI 0.226–0.336) and for carcinogen-induced glioma (0.336, 95% CI 0.196–0.475). An apparent effect in the 9L gliosarcoma model did not reach statistical

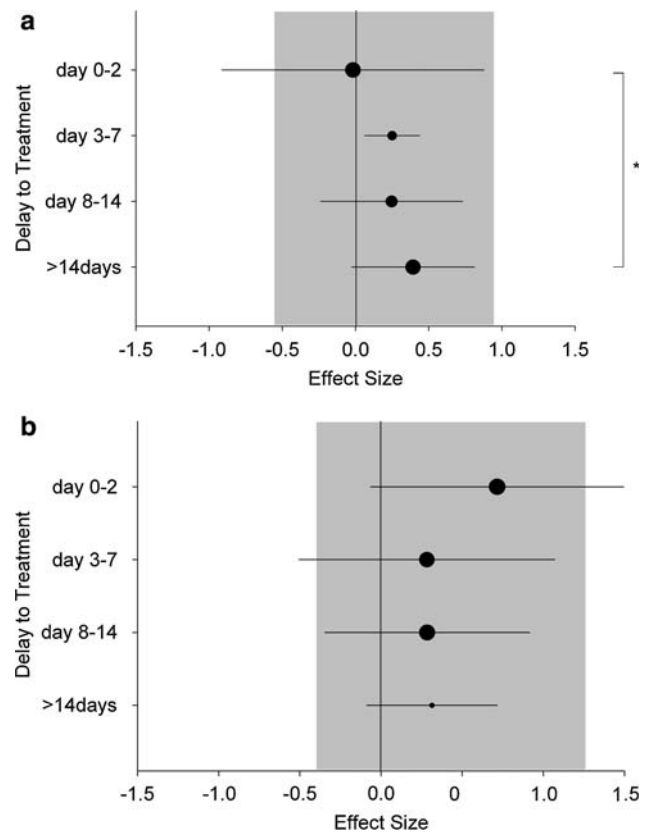


Fig. 3 (a) The effect of time delay of introducing BCNU therapy following intracerebral implantation of glioma cells in experimental models of glioma. Treatment within 2 days of implantation was less effective than delayed therapy. * $P < 0.05$. (b) The effect of time delay of introducing CCNU therapy following intracerebral implantation of glioma cells in experimental models of glioma. Treatment within 2 days of implantation was more effective than delayed therapy, but there were no statistical differences between time points

significance, and BCNU seemed to be without effect in ependymoblastoma (–0.009, 95% CI –0.865 to 0.847) and GL26 and 261 models (0.018, 95% CI –0.791 to 0.828) (Fig. 5a).

For CCNU, there was again an association between efficacy and the method of induction of experimental glioma ($F_{(7,102)} = 3.86$, $P < 0.001$); in contrast with BCNU, CCNU was significantly more effective against tumour cells of murine (0.423, 95% CI –0.386 to 1.272) rather than human (0.085, 95% CI –0.162 to 0.333) origin ($F_{(1,108)} = 5.75$, $P < 0.05$). Significant efficacy was seen in the G XIII (0.246, 95% CI 0.102–0.390) and VDMk 497-P (0.236, 95% CI 0.202–0.271). In contrast with BCNU, substantial efficacy was seen in ependymoblastoma (–0.329, 95% CI –0.742 to 1.401) and GL26 and 261 models (0.601, 95% CI –0.137 to 1.338), although none of these individual estimates reached statistical significance (Fig. 5b).

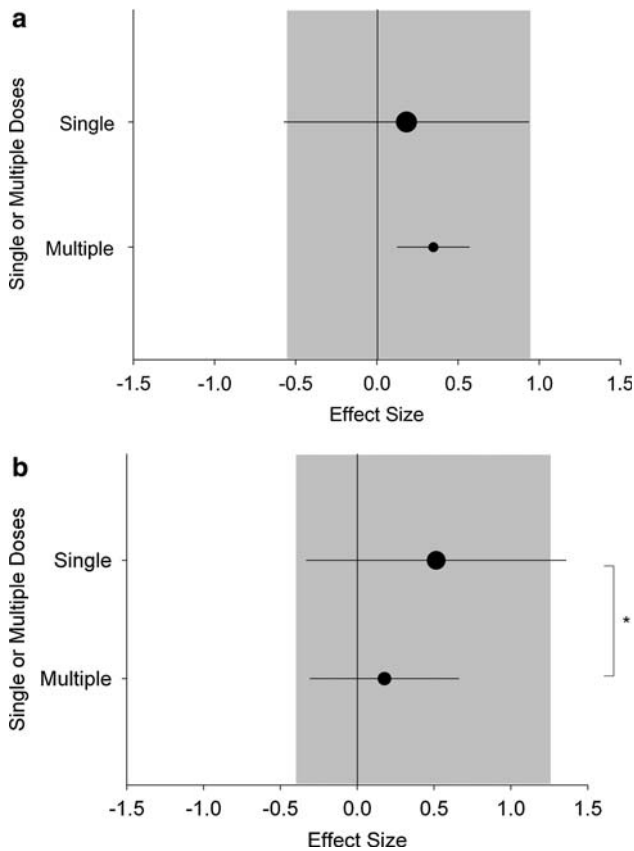


Fig. 4 The effect of multiple versus single dose therapy of either (a) BCNU or (b) CCNU against experimental rodent or murine implantation glioma models. For BCNU there was no difference, whereas for CCNU single dose therapy was more effective than multiple dose therapy. * $P < 0.01$

Animal model and method of drug administration

Efficacy was only reported in rats and mice, and there were no differences in median survival (Rat 0.335, 95% CI -0.181 to 0.853; Mouse 0.304, 95% CI -0.580 to 1.189; Fig. 6). The route of drug delivery did however appear to be important, with greatest efficacy seen with intramuscular treatment (0.751, 95% CI 0.208–1.294), lowest efficacy with intraperitoneal treatment (0.209, 95% CI -0.604 to 1.020) and intermediate efficacy with intravascular (intravenous or intracarotid) treatment (0.402, 95% CI -0.045 to 0.850) [$F_{(2,228)} = 26.7, P < 0.001$; Fig. 6).

Discussion

Numerous in vivo studies have been conducted to determine the efficacy of BCNU and CCNU against experimental glioma. This study has provided a novel quantitative determination of global experimental efficacy for both CCNU and BCNU derived from these experimental studies.

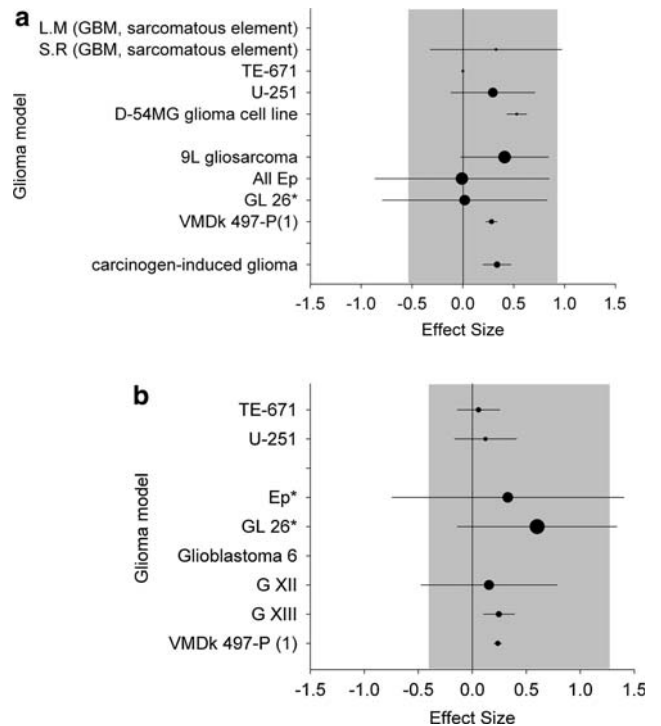


Fig. 5 The influence of experimental implantation glioma model used on efficacy of either (a) BCNU or (b) CCNU. There is a wide range of response to both agents. *Differences between the ependymal cell lines and GL26 to BCNU and CCNU are statistically significant ($P < 0.05$)

Depending on study design characteristics variable efficacy has been demonstrated. Overall global-efficacy estimates for BCNU and CCNU suggest marginal to moderate beneficial effects respectively. The effect sizes are equivalent to a prolongation of median survival for BCNU from 30 to 37 days and for CCNU from 30 to 53 days; however the confidence intervals are very wide and do not exclude a potential detrimental effect.

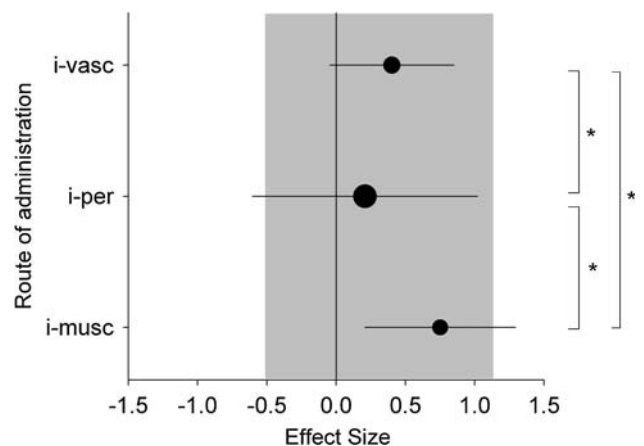


Fig. 6 Effect of route of administration of BCNU and CCNU on efficacy. Intraperitoneal route was least efficacious. *Differences statistically significant, $P < 0.05$

Overall, this analysis suggests that BCNU is less effective than CCNU, but this may be due, at least in part, to there being more data at higher, potentially toxic doses for BCNU. BCNU is significantly more effective when administered in multiple doses than in single doses; indeed, in clinical trials, the potential of multiple-dose BCNU regimens [28, 48] has been recognised, but its systemic complications, have limited its application.

There was no clear relationship between study quality and the estimate of efficacy. In models of other neurological disease, particularly stroke [35, 49], there is increasing recognition of the potential confounding effects of a failure to randomly allocate animals to experimental group, to conceal group allocation from the investigator both during the experiment and when outcome is being measured, and to report sample size calculations. However, in this literature the reporting of such measures to avoid bias is very uncommon, and we believe that it is possible that such methodological weaknesses have led to a substantial overstatement of the efficacy of both BCNU and CCNU. Clearly however, as with the use of the CONSORT guidelines [50] in many human RCTs, these guidelines and suggested internal quality control parameters, post date the publication of this experimental data. They are however indicators for best practice in future experimental studies in neuro-oncology.

We believe this to be the first meta-analysis of animal experiments where the outcome measure has been the change in median survival. We adopted this approach because this was the outcome most commonly reported, but because this is a non-parametric measure with no reported variance it does not lend itself to conventional meta-analytical techniques. We have resolved these difficulties firstly by weighting on the number of animals contributing to the treatment group of each experimental comparison; secondly by transforming median survival to an effect size lying between -1 and 1 ; and thirdly by testing differences between groups using analysis of variance (rather than the more conventional partitioning of heterogeneity) and Tukey's HSD post hoc test.

This approach has a number of potential weaknesses. Firstly, the comparisons which can be made depend entirely on the data available in the published literature, and data presented here are at best associations; they should be viewed as hypothesis generating only. Secondly, there is substantial heterogeneity in these data; it was not possible to conduct a random effects meta-analysis, and the headline figures for efficacy reported here are likely to be misleading. However, the differences in efficacy for BCNU compared with CCNU, and associated with different doses, time of treatment or route of delivery are sufficiently reliable to guide the development of future hypotheses. Thirdly, our analyses are based on published reports of

measures taken to avoid bias, and such measures (for instance randomisation) were taken but not reported, then our analysis will have understated study quality. Finally, the post hoc test used here (Tukey's HSD) becomes more conservative as group size becomes more unequal, and it may be that some important differences between groups have not been identified for this reason.

However, the strengths of this approach are firstly that the systematic search strategy renders this approach less susceptible to citation bias than narrative reviews; the systematic identification of reported measures taken to avoid bias in individual experiments is not confounded by concerns regarding the meta-analytical technique outlined above; and that meta-analytical approach does give some measure of the relative efficacy of different approaches which might be tested in later experiments.

The difficulties and shortcomings of experimental glioma models have been previously recounted [39, 40, 44]. The vast majority of experimental *in vivo* gliomas have many neuropathological shortcomings when compared to human glioma, and the biological features of glioma cell lines vary considerably. These problems are highlighted in the studies included in this review where there were differences in chemotherapeutic responses between human and murine/carcinogen-derived gliomas. Even amongst the human-derived gliomas there were variable chemotherapeutic responses, and in both cases there was also variability of BCNU and CCNU response to specific cell lines. Molecular analysis of chemoresistant and chemosensitive mechanisms within the tumours may potentially give insights into the 'signatures' of tumours that may be specifically sensitive or resistant to chemotherapy.

The early Phase III randomized controlled trials in human malignant glioma contained treatment arms that compared best standard, non-interventional care, and treatment arms involving *inter alia* sole treatment with BCNU, CCNU (see [30]; Table 1). The latter studies are conceptually very similar in design to the experimental studies, although the human studies did involve either surgical biopsy or resection to confirm tumour diagnosis. Both BCNU [28, 51] and CCNU [52, 53] did improve outcome compared with no treatment, although the very low patient numbers preclude any analysis of which agent was better. Following the early Phase III RCTs in human malignant glioma external beam radiotherapy became standard primary post surgical treatment and chemotherapy, usually nitrosourea based, has been used in an adjunctive setting. A meta-analysis of 12 randomised controlled trials that used adjuvant chemotherapy (7 using CCNU and 5 using BCNU based chemotherapy) showed an absolute increase in survivors at 1 year of 6%, and a 2-month increase in median survival time [31]. In contrast to neuroprotective agents in stroke and neurotrauma, where many successful experimental treatments

failed in clinical trials, the translational value of experimental neuro-oncology findings with BCNU and CCNU to clinical trials has therefore been relatively successful. Whether this is the result of serendipity, true effectiveness or rigorous experimental evaluation is debatable.

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