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# Convection enhanced delivery of carmustine to the murine brainstem: A feasibility study

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## HIGHLIGHTS

- No treatment strategy is currently available for diffuse intrinsic pontine glioma (DIPG) and convection enhanced delivery (CED) is a promising technique that could be used to treat these tumors.
- This paper discusses the validation of a new method to study CED in the murine brainstem.
- CED into the murine brainstem using our methods is well tolerated by mice with and without brainstem tumors.
- CED of carmustine is effective in treating two orthotopic murine models of DIPG.
- These results set the foundation for more CED studies in murine DIPG models, to eventually improve therapy for DIPG patients.

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## ABSTRACT

*Background:* Systemic delivery of therapeutic agents remains ineffective against diffuse intrinsic pontine glioma (DIPG), possibly due to an intact blood–brain-barrier (BBB) and to dose-limiting toxicity of systemic chemotherapeutic agents. Convection-enhanced delivery (CED) into the brainstem may provide an effective local delivery alternative for DIPG patients.
*New method:* The aim of this study is to develop a method to perform CED into the murine brainstem and to test this method using the chemotherapeutic agent carmustine (BiCNU). To this end, a newly designed murine CED catheter was tested *in vitro* and *in vivo*. After determination of safety and distribution, mice bearing VUMC-DIPG-3 and E98FM-DIPG brainstem tumors were treated with carmustine dissolved in DW 5% or carmustine dissolved in 10% ethanol.
*Results:* Our results show that CED into the murine brainstem is feasible and well tolerated by mice with and without brainstem tumors. CED of carmustine dissolved in 5% DW increased median survival of mice with VUMC-DIPG-3 and E98FM-DIPG tumors with 35% and 25% respectively. Dissolving carmustine in 10% ethanol further improved survival to 45% in mice with E98FM-DIPG tumors.

*Abbreviations:* DIPG, diffuse intrinsic pontine glioma; BiCNU, 1,3-*bis*-(chloroethyl)-1-nitrosourea; BBB, blood–brain barrier; CED, convection enhanced delivery; GEM, genetically engineered mouse; VUMC, VU University Medical Center; FM, firefly luciferase – mCherry; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; DW, dextrose; PBS, phosphate buffered saline; H&E, hematoxylin and eosin; PET, positron emission tomography; IL-13-PE, interleukin 13–pseudomonas exotoxin; SEM, standard error of the mean.

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*Comparison with existing methods:* Since genetically engineered and primary DIPG models are currently only available in mice, murine CED studies have clear advantages over CED studies in other animals.
*Conclusion:* CED in the murine brainstem can be performed safely, is well tolerated and can be used to study efficacy of chemotherapeutic agents orthotopically. These results set the foundation for more CED studies in murine DIPG models.



## 1. Introduction

Diffuse intrinsic pontine glioma (DIPG) is a fatal brain malignancy in children, for which prognosis has not improved in the last 40 years (Hargrave et al., 2006; Jansen et al., 2012). Although recent *in vitro* studies have shown DIPG cells to be sensitive to both classic chemotherapeutic drugs and novel targeted agents (Veringa et al., 2013), multiple clinical trials have so far been unsuccessful (Jansen et al., 2012). A possible reason for this failure is the inability of therapeutic agents to reach tumor cells, due to a relatively intact blood–brain-barrier (BBB) (Bradley et al., 2008; Smirniotopoulos et al., 2007). The BBB constitutes a physiological barrier to safeguard the central nervous system from exposure to both endogenous and exogenous toxins, thereby also preventing effective delivery of chemotherapy to the tumor parenchyma (Deeken and Löscher, 2007). Therefore, convection-enhanced delivery (CED), a local drug delivery method, may be a promising delivery approach to more effectively treat DIPG patients (Bartels et al., 2011; Hawkins et al., 2011; Song and Lonser, 2008). CED relies on a continuous hydrostatic pressure gradient, which propels therapeutic agents over relevant anatomical volumes, at a speed several orders of magnitude greater than simple diffusion (Bobo et al., 1994). Local drug concentrations achieved by CED can be up to 10,000-fold higher as compared to intravenous drug administration, while minimizing systemic exposure (Groothuis et al., 1999). Because of a wider distribution, CED could be preferred over intrathecal-, intraventricular- and intra-arterial drug administration or polymer-wafer implantation in brain tumor patients (Bidros et al., 2010; Groothuis, 2000; Pardridge, 1997). The use of CED as a treatment strategy for DIPG patients has matured from preclinical studies showing feasibility in rats (Sandberg et al., 2002; Thomale et al., 2009), to safety and distribution studies in non-human primates (Lonser et al., 2002; Yin et al., 2010) and phase I/II clinical trials in children (Murad et al., 2007). To our current knowledge, four studies have been published, describing follow up of five pediatric patients treated with CED in the brainstem, four of which were suffering from a DIPG (Anderson et al., 2013; Barua et al., 2013; Lonser et al., 2007; Saito et al., 2011). These studies have shown CED in DIPG to be feasible and safe but have yet to show a survival benefit. To further improve CED for more (pre-clinical) research is needed. Even though the safety and efficacy of CED in the brainstem has been assessed in rat non-tumor (Sandberg et al., 2002) and non-DIPG brainstem tumor models in rats (Thomale et al., 2009), no study has shown the feasibility of CED in the murine brainstem. Since true primary DIPG-xenograft and genetically engineered models of DIPG are currently only available in mice (Becher et al., 2010; Monje et al., 2011), we decided to conduct a safety and efficacy study of CED in the murine brainstem using the chemotherapeutic agent carmustine (BiCNU, 1,3-*bis*-(chloroethyl)-1-nitrosourea).

Carmustine is an alkylating agent with a clear differential toxicity to pediatric high grade glioma and DIPG cells *in vitro* compared to astrocytes (Veringa et al., 2013). Dose limiting systemic toxicity makes carmustine unsuitable for intravenous therapy in brain tumor patients (Silvani et al., 2009), but its efficacy against glioma cells and the lack of toxicity to astrocytes at clinically relevant concentrations (Veringa et al., 2013), makes it an excellent candidate for local therapy. Currently, carmustine is the only FDA-approved treatment for intracerebral chemotherapy of adult glioblastoma (Buonerba et al., 2011; Hargrave et al., 2006). In addition, interstitial carmustine administration by wafers has shown to be safe in pediatric brain tumor patients (Engelhard, 2000; Sardi et al., 2008)

In this study we evaluated carmustine dissolved in 5% dextrose (DW) as known to be safe in rats. Subsequently, we tested the safety of 10% ethanol administration to the murine brainstem, because dissolving carmustine in 10% ethanol could improve distribution and allows for a better translation into the clinic (Layton et al., 1984). As a final test we studied the *in vivo* efficacy of local delivery of carmustine *via* CED dissolved in both vehicles (5% DW and 10% ethanol). For this purpose we employed our recently developed VUMC-DIPG-3 (Caretti et al., 2014b) model and our previously established E98-Fluc-mCherry (E98-FM) DIPG model (Caretti et al., 2011).

## 2. Materials and methods

### 2.1. Carmustine

For the *in vivo* experiments carmustine (BiCNU), (Bristol-Myers-Squibb, Princeton, NJ), was resuspended to a concentration of 3.3 mg/ml in either 5% DW (pH <4), or 10% ethanol. Carmustine dose was measured *in vitro* before and after the CED procedure using HPLC–UV in both vehicles.

### 2.2. Convection-enhanced delivery in vitro

The CED-system was tested *in vitro* by performing CED of trypan blue in 0.6% agarose gel, which has been previously described as a reliable model to simulate CED in the brain parenchyma (Chen et al., 2004). CED was performed at a speed of 0.5 μl/min for 30 min. Total infusion volume was 15 μl. Five minutes after the end of the procedure, the inner cannula was withdrawn and after 1 min the guide was also withdrawn at a speed of 1 mm/min. Distribution and backflow were assessed by observing trypan blue distribution in the agarose gel.

### 2.3. Animals used for convection-enhanced delivery experiments

Animal experiments were performed in accordance with the Dutch law on animal experimentation and the protocol was approved by the committee on animal experimentation of the VU University Medical Center (VUMC). All tumor models (E98FM-DIPG, total $n = 31$, VUMC-DIPG 3, total $n = 12$) were established in immune deficient 6-week-old athymic nude-foxn1$^{nu}$ mice to allow for adequate engraftment. Toxicity studies of 10% ethanol infusion (total $n = 15$) were performed on 6-week-old balb/c mice with an intact immune system to study the full scope of possible tissue reactions. All mice were housed under specific pathogen-free conditions in a 12-h light–dark cycle and were offered food and water *ad libitum*. Weights were measured and clinical scores were assigned daily after the CED procedure (toxicity and efficacy studies). Clinical scores ranged from 0 to 4 and referred to 0: normal active behavior, 1: subtle inactivity or subtle neurological symptoms, 2: mild to moderate inactivity or neurological symptoms, 3: severe