Published in final edited form as:

J Neurosurg. 2012 December ; 117(6): 1150–1154. doi:10.3171/2012.9.JNS12506.

Comparison of pulsed versus continuous convective flow for central nervous system tissue perfusion:

Laboratory investigation

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Abstract

Object—Although pulsatile and continuous infusion paradigms have been described for convective delivery of drugs, the effectiveness and properties of each flow paradigm properties of pulsatile and continuous convective infusion paradigms, the authors compared these convective flow methods in the gray and white matter of primates.

Methods—Six primates (*Macaca mulatta*) underwent convective infusion of Gd-DPTA (5 mM) into the cerebral gray matter (thalamus) or white matter (frontal lobe) using pulsed (intermittent pulses of 15 μ l/min) or continuous (1 μ l/min) convective flow. Results were assessed by clinical MRI and histological analyses.

Results—Distribution of Gd-DTPA infusate in gray and white matter by pulsed and continuous flow was clearly identified using MRI, which revealed that both convective flow methods demonstrated an increase in the volume of distribution (Vd) with increasing volume of infusion (Vi) in the surrounding gray and white matter. Although the mean ($\pm$ SD) gray matter Vd:Vi ratio for the pulsed infusions (4.2 ± 0.5) was significantly lower than the mean Vd:Vi ratio for continuous infusions (5.4 ± 0.5 ; a 22% difference [$p = 0.0006$]), the difference between pulsed (3.8 ± 0.4) and continuous (4.3 ± 1.2) infusions in white matter was not significantly different ($p = 0.3$). Pulsed infusions were associated with more leakback ($12.3\% \pm 6.4\%$ of Vi) than continuous infusions ($3.9\% \pm 7.8\%$), although this difference was not significant ($p = 0.2$). All animals tolerated the infusions and there was no injury at the infusion sites.

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Disclosure

This work was supported by the intramural program of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health. Dr. Lonser holds a patent with the National Institutes of Health.

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. Author contributions to the study and manuscript preparation include the following. Conception and design: all authors. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: Lonser, Nduom. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Lonser. Statistical analysis: Lonser, Nduom. Administrative/technical/material support: all authors. Study supervision: all authors.

Conclusions—Although pulsed and continuous infusion flow paradigms can be safely and effectively used for convective delivery into both gray and white matter, continuous infusion is associated with a higher Vd:Vi ratio than pulsatile infusion in gray matter. High rates of infusion (15 μ l/min) can be used to deliver infusate without any significant leakback and without any clinical or histological evidence of injury.

Keywords

pulsatile flow; continuous flow; convection-enhanced delivery; drug delivery; nervous system; *Macaca mulatta*

Pharmacological agents can be delivered to the extracellular spaces of nervous system parenchyma behind the blood-brain barrier using CED. Convec-employs a small hydrostatic pressure gradient.³ Because convective properties are based on bulk flow, CED of infusate in the CNS is possible using either pulsed or continuous infusion paradigms. Although prior laboratory research and clinical studies have employed both patterns of infusate flow for convective delivery, there are no data that compare the effectiveness and clinical properties of both methods.^{11,13,15,17}

To determine the effectiveness and clinical properties of pulsed and continuous convective flow in the CNS, we infused primate brains (white and gray matter) with Gd-DTPA using either pulsed or continuous convective infusion while performing real-time MRI.

Methods

Animals

Animal studies were performed under an institutional approved animal care and use protocol. Four adult non-human primates (*Macaca mulatta*) underwent infusion to the bifrontal white matter or to the bilateral thalami. Two primates (*Macaca mulatta*) underwent infusion to both the bilateral thalamic gray matter and bilateral frontal white matter.

Preparation of the MRI Agent

Gadolinium-DTPA (Bayer HealthCare Pharmaceuticals, Inc.) was diluted to a 5 mM concentration in sterile 1X phosphate-buffered saline (KD Medical) for all infusions.

Convective Infusion

Procedures were performed under general anesthesia with monitoring. After placing the animal head in a stereotactic head frame (Crist Instrument Company), bilateral bur holes over the thalami and/or frontal lobe white matter were drilled.^{2,7} Targeting array pedestals were placed over the bur holes and then fixed to the cranium with Triad Gel adhesive (DENTSPLY International, Inc.) and nylon screws.² A single-step infusion cannula made of fused silica composed of a 22-gauge outer cannula and a 27-gauge inner cannula that protruded 3 mm beyond the outer cannula (Bankiewicz Laboratory) was placed through the affixed array to the target for each infusion.⁸

Infusion Paradigms

Bilateral thalamic gray or frontal white matter infusions were performed according to the following infusion paradigms. Continuous flow infusions were carried out at 1 $\mu\text{l}/\text{min}$. Pulsed infusions were performed at 15 $\mu\text{l}/\text{min}$ for 24 30 secs every 7 7.5 mins with a basal rate of 0.13 $\mu\text{l}/\text{min}$ between infusion pulses. The pulsed rate of 15 $\mu\text{l}/\text{min}$ was chosen as it was significantly faster than the rate of infusion used in continuous infusions described in the literature but not so high as to risk having significant leakback. The pulsed sequence was designed so that an identical V_i would be delivered on the pulsed side as on the continuous side at experiment completion.

Imaging of Infusate Distribution

After MRI confirmed the accurate placement of cannulas, high-resolution T1-weighted MRI sequences were obtained throughout the infusion using a 3-T MRI scanner, as previously described.² There was no variation in the timing of the MRI of each infusion. The final MR images were obtained beginning within 4 mins of completion of the infusion in each case.

Magnetic Resonance Imaging Analysis

The MRI of infusion V_d was quantified (MEDx version 3.44, Medical Numerics) using a segmentation threshold (infusate intensity 10% above mean T1-weighted signal intensity of noninfused tissue) as previously described.¹² The V_d to V_i ratios were calculated for each infusion. Leakback was back along the cannula outside of the spherical infusate distribution.

Clinical and Histological Analysis

Animals who had 2 pedestals placed were observed for neurological compromise after infusion. The 2 animals with 4 pedestals were killed immediately after infusion for histopathological analysis.

Statistical Analysis

Statistical analysis was performed as described in the text using Microsoft Excel 2007. Values are expressed as mean $\pm$ SD.

Results

Imaging of Infusions

Perfusion of the thalamic gray and frontal white matter with 5 mM Gd-DTPA was clearly identified (Fig. 1). Perfused tissue V_d increased in gray and white matter, with an increased V_i using either pulsed or continuous convective flow paradigms. Infusions were contained within their respective tissue types (gray or white matter) and stopped before overflow into surrounding adjacent tissues. Leakback could be identified along the needle track using both pulsed and continuous flow paradigms and in gray and white matter (Fig. 2).

Overall, the leakback (as a percentage of total V_d) identified along the cannula tracks was not significantly different ($p = 0.2$, Student t-test) between the pulsed ($12.3\% \pm 6.4\%$ of V_i) and continuous ($3.9\% \pm 7.8\%$) flow infusions. In gray matter, mean leakback for pulsed

infusions was not significantly higher ($9.1\% \pm 7.7\%$) than mean leakback for continuous infusions ($2.9\% \pm 5.3\%$) ($p = 0.1$, Student t-test). Similarly, in white matter, mean leakback for pulsed infusions was not significantly higher than continuous infusions ($15.5\% \pm 22.7\%$ vs $4.9\% \pm 9.9\%$, respectively; $p = 0.5$, Student t-test). Leakback between all white matter infusions compared with those in gray matter was not significantly different ($10.2\% \pm 17.1\%$ vs $6.0\% \pm 6.9\%$, respectively; $p = 0.6$, Student t-test).

Analysis of Imaging

Both Gray and White Matter Infusions—Overall, the mean Vd:Vi for pulsed infusions (4.0 ± 0.5) was significantly less than the mean Vd:Vi for continuous CED infusions (4.8 ± 1.0 , an 18% difference; $p = 0.007$, Student t-test) (Table 1; Fig. 3). Mean Vd:Vi for the pulsed thalamic gray matter infusions (4.2 ± 0.5) was significantly lower than continuous thalamic gray matter infusions (5.4 ± 0.5 , a 22% difference; $p = 0.0006$, Student t-test). Mean Vd:Vi for pulsed frontal white matter infusions (3.8 ± 0.4) was similar to continuous frontal white matter infusions (4.3 ± 1.2 , a 12% difference; $p = 0.3$, Student t-test).

Safety

Four animals were observed for neurological compromise after infusion (mean observation period 12 days, range 8–22 days) before being euthanized. The 2 animals killed immediately after infusion underwent histopathological analysis. No adverse effects occurred in the animals that underwent clinical observation after the pulsed or continuous infusions in the gray and/or white matter. No evidence of toxicity or trauma was seen on histological analysis of the pulsatile or continuous infusion regions in either the gray or white matter.

Discussion

Previous Studies

Previous studies have not compared the effects of pulsatile and continuous CED to the CNS. Nevertheless, groups have used pulsed or continuous convective delivery of a variety of putative therapeutic agents via intraparenchymal infusion catheters/cannulas.^{1,4,6,10,16} Review of previous clinical trials involving CED reveals that continuous infusions were most frequently used in most studies. The rates used in these studies were typically between 1 $\mu\text{L}/\text{min}$ and 12.5 $\mu\text{L}/\text{min}$.^{5,9,14,19} In a long-term infusion trial for Parkinson disease, a low basal rate of 0.03 $\mu\text{L}/\text{min}$ with pulses of 21.3 μL delivered over 117 secs every 6 hours (10.9 $\mu\text{L}/\text{min}$) was used in patients for 24 weeks, but the Vd was not tracked.¹⁶ We studied the practicability and properties of pulsatile and continuous perfusion of gray and white matter using Gd-DTPA and real-time MRI.

Current Study

Imaging—Previous work has demonstrated that 5 mM Gd-DTPA provides a reliable representation of the Vd of infusate over a wide range of molecular weights in the primate brain.² Consistent with previous data, 5 mM Gd-DTPA provided clear contrast between infused and non-infused gray and white matter regions on real-time MRI for both pulsatile and continuous infusion paradigms. Consistent with previous studies, the clear distinction between perfused and nonperfused regions permitted accurate segmentation analysis of the

perfused tissue Vd on T1-weighted sequences, as well as quantification of leak-back along the needle track.

Pulsed Versus Continuous Flow—Both pulsed and continuous infusions reliably delivered infusate to the specified target. Overall, consistent with previous studies the Vd:Vi ratio was approximately 5.^{2,18} There was a significant reduction in Vd for pulsed infusion compared with continuous infusion (4.2 ± 0.5 vs 5.4 ± 0.5 , respectively; $p = 0.0006$, Student t-test) for gray matter infusions. However, although the Vd:Vi ratio for pulsatile infusions was smaller than continuous white matter infusions, the difference was not significantly different (3.8 ± 0.4 vs 4.3 ± 1.2 , respectively; $p = 0.3$, Student t-test).

A likely explanation for the difference between pulsatile and continuous infusion Vd:Vi is the increased association of infusate leakback along the cannula track. While these differences were found to be nonsignificant, there was nevertheless an 8.4% difference between the 2 types of infusion with pulsatile infusions associated with more leakback. There was 6.2% more leakback in gray matter and 10.5% more leakback in white matter associated with pulsatile infusion. The volume of infusate lost to leakback was not distributed to interstitial spaces where it would distribute over approximately 5-fold greater tissue area (that is, a Vd:Vi ratio of 5). Because infusions into either gray or white matter were greater than 1 cm into the brain parenchyma, it would be unlikely that the depth of infusion cannula would account for any differences in leakback (that is, more superficial infusions may be associated with more leakback) between gray and white matter. Nevertheless, it is possible that leakback would be significant with very superficial infusions (that is, cortical infusions) that have minimal tissue around the cannula.

Safety—The 4 animals that were not immediately killed did not exhibit any neurological symptoms during their postinfusion observation period. Furthermore, there were no histopathological findings that would indicate any damage to the brain related to the higher rate of infusion used during the pulsatile infusions compared with continuous infusions. Pulsatile infusion rates of 15 μ l/min were well tolerated by the primates, indicating that higher rates of infusion may be well tolerated in a clinical setting.

Conclusions

Although pulsed and continuous infusion flow paradigms can be safely and effectively used for convective delivery in gray and white matter, continuous infusion is associated with a higher Vd:Vi ratio than pulsatile infusion in gray matter. High rates of infusion (15 μ l/min) can be used to deliver infusate without significant leakback and without evidence of clinical or histological injury.

Abbreviations used in this paper

CED	convection-enhanced delivery
Vd	volume of distribution
Vi	volume of infusion

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Fig. 1. Coronal T1-weighted MR image demonstrating the pulsed (*left side*) and continuous (*right side*) infusion of Gd-DTPA (5 mM; total Vi per side 34 μ l). The Vd on the pulsed side was 143.4 mm³ and on the continuous infusion side was 186.7 mm³.

Fig. 2.
Coronal T1-weighted MR image of a pulsed infusion into the right frontal white matter.
Leakback was noted along the catheter (*box*).

Fig. 3.

Bar graphs demonstrating the differences between the mean Vd:Vi ratios for pulsed and continuous infusions. *Dark gray bars* represent pulsed infusions and *light gray bars* represent continuous infusions. **A:** There was a significant difference in the mean Vd:Vi ratios comparing pulsed infusions and continuous infusions when white matter and gray matter infusions were taken together (continuous 4.8 ± 1.0 and pulsed 4.0 ± 0.5 , an 18% difference; $p = 0.007$, Student t-test). **B:** There was also a significant reduction in the Vd:Vi ratio of pulsed infusions versus continuous infusions when examining gray matter infusions alone (4.2 ± 0.5 vs 5.4 ± 0.5 , respectively; $p = 0.0006$, Student t-test). **C:** There was a reduction in the Vd:Vi ratio of pulsed infusions compared with continuous infusions in white matter, but this difference was not significant (3.8 ± 0.4 vs 4.3 ± 1.2 , respectively; $p = 0.3$, Student t-test).

TABLE 1

Distribution of pulsed and continuous Gd-DTPA infusions

Infusion No.	Target	Infusion Type	Vol Infused (μl)	Vd:Vi Ratio
1	frontal white matter	pulsed	30	3.6
	frontal white matter	CED	30	4.1
2	frontal white matter	pulsed	30	3.6
	frontal white matter	CED	30	4.2
3	frontal white matter	pulsed	30	4.4
	frontal white matter	CED	30	5.8
4	frontal white matter	pulsed	30	3.5
	frontal white matter	CED	30	3.0
5	thalamus	pulsed	34	4.9
	thalamus	CED	34	6.0
6	thalamus	pulsed	34	4.0
	thalamus	CED	34	5.0
7	thalamus	pulsed	34	4.2
	thalamus	CED	34	5.5
8	thalamus	pulsed	34	3.8
	thalamus	CED	34	5.1