Real-time in vivo imaging of the convective distribution of a low-molecular-weight tracer

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Object. Convection-enhanced delivery (CED) is increasingly used to distribute therapeutic agents to locations in the central nervous system. The optimal application of convective distribution of various agents requires the development of imaging tracers to monitor CED in vivo in real time. The authors examined the safety and utility of an iodine-based low-molecular-weight surrogate tracer for computerized tomography (CT) scanning during CED.

Methods. Various volumes (total volume range 90–150 μl) of iopamidol (MW 777 D) were delivered to the cerebral white matter of four primates (Macaca mulatta) by using CED. The distribution of this imaging tracer was determined by in vivo real-time and postinfusion CT scanning (≤ 5 days after infusion [one animal]) as well as by quantitative autoradiography ([14C]-sucrose [all animals] and [14C]-dextran [one animal]), and compared with a mathematical model. Clinical observation (≤ 5 months) and histopathological analyses were used to evaluate the safety and toxicity of the tracer delivery. Real-time CT scanning of the tracer during infusion revealed a clearly definable region of perfusion. The volume of distribution \( V_d \) increased linearly \( (r^2 = 0.97) \) with an increasing volume of infusion \( V_i \). The overall \( V_d/V_i \) ratio was 4.1 ± 0.3 (mean ± standard deviation) and the distribution of infusate was homogeneous. Quantitative autoradiography confirmed the accuracy of the imaged distribution for a small (sucrose, MW 359 D) and a large (dextran, MW 70 kD) molecule. The distribution of the infusate was identifiable up to 72 hours after infusion. There was no clinical or histopathological evidence of toxicity in any animal.

Conclusions. Real-time in vivo CT scanning of CED of iopamidol appears to be safe, feasible, and suitable for monitoring convective delivery of drugs with certain features and low infusion volumes.

Key Words • central nervous system • computerized tomography • convection-enhanced delivery • drug delivery • Macaca mulatta

Convection-enhanced delivery is increasingly used to distribute therapeutic agents to locations in the central nervous system. In CED, a small hydrostatic pressure differential imposed by a syringe pump to distribute infusate directly to small or large regions of the CNS is used in a safe, reliable, targeted, and homogeneous manner that bypasses the blood–nervous system barrier. Convection-enhanced delivery has a number of advantages over existing delivery techniques and has permitted the development of a number of new treatment approaches in preclinical and clinical studies. Nevertheless, the optimal use of convective distribution of a therapeutic infusate requires the development of imaging tracers to monitor drug distribution during CED.

The development of surrogate tracers for use in CT scanning in conjunction with CED will be useful for several reasons. Computerized tomography scanning is widely available and easily accessible. The scans can be rapidly obtained, thus minimizing image acquisition time and enhancing the ease and value of sequential imaging. High-resolution images together with an appropriately matched tracer can be used to assess the precise anatomical location and volume of drug distribution. This will be an important feature in determining the efficacy of new treatment approaches using CED.

Nguyen, et al., previously demonstrated the successful use of iopanoic acid–labeled albumin as a surrogate tracer for CT scanning of HMW compounds; however, HMW tracers may not reliably reflect the distribution of smaller therapeutic agents. Because of the advantages associated with the development of an imaging tracer to be used in combination with CED of various smaller therapeutic agents, we investigated the use of an LMW surrogate marker for real-time in vivo CT scanning during convective infusion. To determine the safety, parameters, and utility of CED of an iodine-based surrogate tracer for CT scanning, we perfused the cerebral white matter of primates with iopamidol and performed real-time and postinfusion CT scan-
Real-time in vivo imaging of CED of an LMW tracer

Materials and Methods

Tracer Use for CT Scanning

Iopamidol (MW 777 D, concentration 408 mg/ml; Isovue-M200; Bracco Diagnostics, Princeton, NJ), containing 20% organically bound iodine (200 mg/ml; pH 6.5–7.5) was the imaging tracer used for distribution in this study.

Toxicity Trial of Iopamidol in Rats

All animal investigations were conducted in accordance with the National Institutes of Health guidelines on the use of animals in research and were approved by the Animal Care and Use Committee of the National Institute of Neurological Disorders and Stroke. Six adult male Sprague–Dawley rats (each weighing between 300 and 400 g) were anesthetized by intraperitoneal administration of 80 mg/kg ketamine and 10 mg/kg xylazine, and placed in a stereotactic frame (Kopf Instruments, Tujunga, CA). A midsagittal scalp incision was made to the level of the skull and a burr hole was placed over the right frontal region. A 32-gauge cannula attached to a 25-µl Hamilton syringe (Thompson Scientific Instruments, Clear Brook, VA) filled with iopamidol was stereotactically placed in the right striatum. The coordinates for placement of the cannula tip in the striatum were 0.5 mm anterior to the bregma, 2.6 mm right of midline, and 5 mm below the dura mater. To distribute the infusate (iopamidol) by convection, we used a noncompliant, gas-tight infusion apparatus consisting of a syringe pump (Harvard Apparatus, South Natick, MA) that generates a continuous pressure gradient, which is transmitted through a hydraulic drive attached to the infusate syringe plunger.12 Using this system, 10 µl of iopamidol (408 mg/ml) was delivered at a rate of 0.5 µl/minute into the striatum. After completion of the infusion, the animals were observed daily for clinical deficits and were killed at the end of the observation period (3 days, three animals and 4 weeks, three animals) into the striatum. After completion of the infusion, the animals were observed daily for clinical deficits and were killed at the end of the observation period (3 days, three animals and 4 weeks, three animals). After the animals had been killed their brains were immediately removed and frozen at −70°C. The brains were later cut coronally into 20-µm-thick serial sections (throughout the entire brain) on a cryostat at −18 to −20°C and stained with H & E, Luxol fast blue, and Nissl.

Infusion of Iopamidol in Primates

Surgery. Applying the CED technique, iopamidol was delivered to various regions of the cerebral hemispheric white matter of four adult nonhuman primates (Macaca mulatta) (Table 1). After induction of sedation, the animals underwent endotracheal intubation and induction of general anesthesia with inhaled halothane (1–4%). Each primate’s temperature, heart rate, oxygen saturation, electrocardiographic signals, and end-tidal PCO2 were monitored. The head of each animal was secured in a stereotactic frame (Kopf Instruments). Using a sterile technique, a midline scalp incision was made from the anterior to the posterior aspect of the vertex, and self-retaining retractors were used to expose the underlying skull. One or two 1-cm burr holes were made over the stereotactically determined entry point(s) and the underlying dura mater was incised. A silicate infusion cannula (Plastics One, Inc., Roanoke, VA; outer diameter 0.36 mm, inner diameter 0.15 mm) was stereotactically placed through the dural opening and positioned with its tip at the predetermined target. The silicate infusion cannula was connected to the vinyl tubing containing the infusate (Plastics One, Inc.; outer diameter 1.3 mm, inner diameter 0.6 mm) via a side port and secured to the skull with methylmethacrylate. The vinyl tubing was then tunneled subcutaneously under the scalp and posterior nuchal skin, and exited through the skin between the scalpula in the midline. A 2-ml syringe containing the infusate was connected to the vinyl tubing and placed in a microinfusion pump (MiniMed Technologies, Sylmar, CA). The pump was secured in a custom-made jacket (Lomir Biomedical, Inc., Malone, NY). A separate additional cannula, vinyl subcutaneous tubing, and pump were placed in a similar manner in animals in which two infusions were performed (Table 1). After the infusion apparatus had been placed, the wound was closed and the animal was brought to the imaging center and secured to the CT scanner.

Real-Time CT Scanning. Coronal CT scans were obtained to determine the precise location of the cannula(e) before any infusion was initiated. Once the cannula position had been confirmed, the infusions had been started, and real-time coronal (slice thickness 1 mm, 1-mm spacing) CT scans were obtained at 15- to 60-minute intervals while the animal lay prone. In all instances the initial rate of infusion was 0.5 µl/minute; this rate was maintained for 30 to 60 minutes and then increased to 1 to 3.5 µl/minute. The CT scans were analyzed on a Sun Workstation (Sun Microsystems, Inc., Palo Alto, CA). The Vr was calculated using a threshold for segmentation consisting of 10% of the value obtained from the region of interest containing the maximal optical density. To determine the homogeneity of infusion over the infused Vr line profiles were obtained through the center of the infusion as seen on coronal images.

Postinfusion CT Scanning. One primate underwent daily CT scanning starting on postinfusion Day 1 and continuing until Day 5. Coronal (slice thickness 1 mm, 1-mm spacing) CT scans were obtained while the animal lay prone.

Clinical Evaluation. With the exception of the primate used for the QAR analysis, which was killed immediately after infusion completion, the animals were observed daily for medical problems or neurological deficits during the study (≤ 5 months). Three surviving primates were also observed by performing postoperative videotaping.

Quantitative Autoradiography Analysis. Quantitative autoradiography is a highly accurate method of determining spatial distributions of radiolabeled molecules within tissue. Radioisotope emissions from infused tissue sections release energy onto a superimposed photographic film, forming density images that can be quantified. To determine precisely the spatial distribution of LMW and HMW radiolabeled compounds and to determine the accuracy of iopamidol as a surrogate tracer for these variously sized compounds, we performed QAR on tissue sections obtained from a primate that underwent bilateral confusions of iopamidol (concentration 408 mg/ml) with 14C-sucrose (MW 359 D; Sigma Chemical Co., St. Louis, MO) to the right frontal white matter and 14C-dextran (MW 70 kD; Sigma Chemical Co.) to the left frontal white matter. The targeted

TABLE 1

Summary of primates infused with iopamidol

<table>
<thead>
<tr>
<th>Primate No.</th>
<th>Region(s) Perfused</th>
<th>No. of Cannulae</th>
<th>Total Vr (µl)</th>
<th>QAR</th>
<th>Mean Vr/Vr Ratio*</th>
<th>Observation Period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rt frontal</td>
<td>1</td>
<td>150</td>
<td>no</td>
<td>4.1</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>rt frontal</td>
<td>2</td>
<td>97</td>
<td>yes</td>
<td>4.7</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>lt frontal</td>
<td>2</td>
<td>90</td>
<td>yes</td>
<td>4.7</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>rt parietal</td>
<td>1</td>
<td>90</td>
<td>no</td>
<td>3.8</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>rt temporal</td>
<td>1</td>
<td>142</td>
<td>no</td>
<td>4.1</td>
<td>150</td>
</tr>
</tbody>
</table>

* The Vr of iopamidol was determined using CT scanning. The mean Vr/Vr is the average value derived from all CT scans obtained at each scanning interval during the infusion.
range of radioactivity was 400 to 800 nCi/g of brain. The animal was killed immediately after completion of the infusion, and the brain was harvested and sectioned as described later. Twenty-micrometer-thick sections and \(^{14}C\) standards (range 0.002–35 \(\mu\)Ci/g; American Radiolabeled Chemicals, Inc., St. Louis, MO) were exposed on BAS-MS imaging plates (Fuji Medical Systems, Stamford, CT), and developed using a BAS-5000 Bio-Imaging Analyzer (Fuji Medical Systems). The area of distribution on each slide was measured with the aid of the Image Gauge (version 3.45) software program (Fuji Medical Systems). A threshold for segmentation of 10% of the value obtained from the region of interest containing the maximal optical density was used to determine the area of distribution. The \(V_d\) was calculated by summing the areas and multiplying this sum by 0.1 mm. To determine the homogeneity of infusion over the infused \(V_d\), line profiles were obtained through the center of the infusion, as determined using autoradiography.

**Histological Analysis.** The animals were killed by an intravenous overdose of pentobarbital (90 mg/kg) at the completion of the observation period (Table 1). All animals were perfused with phosphate-buffered saline followed by 4% paraformaldehyde. Their brains were immediately removed and frozen at \(-70\)˚C. Twenty-micrometer-thick coronal sections throughout the brain were cut on a cryostat at \(-18\) to \(-20\)˚C. The sections were stained using H & E, Luxol fast blue, and Nissl.

**Statistical Analysis.** Statistical analysis was performed using commercially available software. Specific statistical tests were used as defined elsewhere in the text.

**Results**

**Rat Toxicity Studies**

None of the six rodents in which the CED technique was used to deliver iopamidol (10 \(\mu\)l) to the right striatum had clinical evidence of toxicity during a short-term (3 days, three animals) or long-term (4 weeks, three animals) observation. A histological analysis of tissues excised from these six animals revealed normal tissue architecture with minimal gliosis (25-\(\mu\)m radius) that was limited to the region immediately surrounding the cannula tract.

**Primate Infusion Studies**

**Real-Time CT Scanning.** Computerized tomography scanning performed at 15- to 60-minute intervals throughout the infusions revealed that the anatomical region infused with iopamidol was clearly distinguishable from the surrounding noninfused tissue (Fig. 1). The region surrounding the cannula tip steadily filled with contrast material, with an increasing \(V_i\) (Fig. 1).

Volumetric analysis of the infused region at various time points throughout the infusion revealed that the \(V_i\) increased linearly \((r^2 = 0.97)\) with the increasing \(V_i\) (Fig. 2). The overall \(V_i/V_d\) ratio was 4.1 ± 0.7 (mean ± standard deviation). The concentration across the infused region was homogeneous. Cross-sectional line profiles performed...
across the infused region formed a square-shaped distribu-
tion pattern with a relatively steep dropoff of concentration
at the edges (Fig. 3).

**Postinfusion CT Scanning.** Postinfusion scanning per-
fomed in one animal (Primate 1) daily up to 5 days af-
after completing the infusion revealed an increase in the Vd
on postinfusion Day 1. A quantitative assessment of the
increase was limited by the reduction in imaging intensity
of the portion of the Vd that was below the segmentation
threshold. This reduction remained stable until the density
of the region perfused by iopamidol reached the density of
surrounding tissue by postinfusion Day 3 (Fig. 4). Cross-
sectional line profiles performed through the infused region
on postinfusion Day 1 revealed a flattening of the concentra-
tion distribution pattern and a loss of the square-shaped
profile that was observed on CT scans during the infusion
(Fig. 3).

**Clinical Evaluation.** Serial clinical examinations of three
primates after infusion showed no evidence of toxicity for
the duration of observation, which lasted up to 5 months
(Table 1). One animal (Primate 2) was killed immediate-
lly after infusion so that we could perform a tissue QAR
analysis.

**Quantitative Autoradiography Analysis.** Imaging and the
QAR tissue analysis (Primate 2) of the coinfusion of io-
pamidol and either 14C-sucrose (on the right side) or 14C-
dextran (on the left side) into the frontal white matter re-
vealed a close correlation between the Vd measured on CT
scans (right side, 430 mm³; left side, 480 mm³) and the Vd
determined by performing QAR (sucrose on the right side,
515 mm³; dextran on the left side, 516 mm³; Table 2). The
QAR patterns and regions of distribution for the labeled su-
crose and dextran matched those observed on the CT scans
(Fig. 5). The Vd's obtained in Primate 2 by imaging were
lower than the Vd's measured by QAR—19.7% lower for su-
crose and 7.5% lower for dextran—but the differences were
not significant, given an estimated coefficient of variation
of 0.17 for the Vd derived from CT scanning and a simi-
lar value for the Vd derived from QAR (Table 2). Similar to
the CT analysis of homogeneity, cross-sectional line profiles
across the infused region on QAR formed a square-
shaped distribution pattern with a steep dropoff of concentra-
tion at the edges (Fig. 6).

**Histological Analysis.** A gross examination of the brain re-
vealed normal weight and no evidence of edema. Sections
throughout the region of infusion prepared using Luxol fast
blue, H & E, and Nissl stains revealed a normal tissue archi-
tecture. Gliosis was limited to the region immediately sur-
rounding the cannula track (within a 50-μm radius).

### Table 2

<table>
<thead>
<tr>
<th>Molecule</th>
<th>MW (D)</th>
<th>Vd (µl)</th>
<th>CT Scanning</th>
<th>QAR</th>
<th>% Difference Btw Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>sucrose</td>
<td>359</td>
<td>97</td>
<td>430</td>
<td>515</td>
<td>19.7</td>
</tr>
<tr>
<td>dextran</td>
<td>70,000</td>
<td>97</td>
<td>480</td>
<td>516</td>
<td>7.5</td>
</tr>
</tbody>
</table>

* Data were obtained in Primate 2 (see Table 1). The Vd of iopamidol was
determined using CT scanning, and the Vd's of the individual agents was
determined using QAR.
Convection-Enhanced Delivery

Convection-enhanced delivery relies on bulk flow that is driven by a small pressure gradient to distribute molecules within the interstitial spaces of the CNS. Unlike intraventricular, intrathecal, or polymer implant delivery techniques, which rely on diffusion, convection is not limited by the infusate’s molecular weight, concentration, or diffusivity. Moreover, because convective delivery directly distributes molecules within the parenchyma, it can be used to target selected regions of the CNS in a manner that bypasses the blood–nervous system barrier, which limits the distribution and efficacy of systemically delivered agents. The properties of convective delivery permit the homogeneous distribution of small and large molecules over clinically relevant volumes in a safe and reproducible manner within peripheral nerves, the spinal cord, the brainstem, and the brain.

Previously, we examined the feasibility, properties, and safety of convective distribution of HMW surrogate imaging tracers for magnetic resonance and CT imaging. These studies showed that albumin labeled with Gd (for magnetic resonance imaging) or iopanoic acid (for CT scanning) could be used to monitor accurately the convective delivery of a large protein (14C-albumin; MW 72 kD). Because both these tracers are of HMW and are not significantly affected by diffusive forces, they may not accurately track LMW substances, particularly during long infusions during which diffusion can influence distribution at the leading edges of the Vd. Here an LMW, commercially available tracer suitable for CT scanning was examined using in vivo real-time monitoring, and the distribution of the tracer was compared with the distribution of small and large molecules within the primate brain.

Current Study

Iopamidol. Iopamidol is a nonionic, organic, iodine-based contrast agent suitable for use in CT scanning. It was chosen as an imaging surrogate tracer for CED for several reasons. Because iopamidol contains organically bound iodine, it can be easily and reliably imaged during CT scanning. It has been used extensively for CT scanning, particularly myelography, and its safety when used in the CNS has been well-documented, even in patients who previously have displayed intolerance to iodine derivatives. Because iopamidol has a low octanol–water coefficient (logP value −2.42), its transfer across the blood–brain barrier into the vascular compartment is minimal. This property should allow its distribution during CED to approximate closely the convective distribution of therapeutic agents that do not cross the blood–nervous system barrier, a feature of the most suitable agents for CED in the CNS.

Real-Time Imaging Characteristics. Computerized tomography scanning during iopamidol infusion revealed a well-defined Vd that was easily distinguished from the surrounding brain parenchyma. The region surrounding the tip of the cannula was filled steadily with an increasing Vd (Fig. 1). During infusion into the centrum semiovale, tracer distribution was confined to the white matter, reflecting the preferential flow of the agent and the reduced resistance to the flow of fluid along the longitudinal axes of white matter tracts, relative to gray matter. This, in turn, was reflected as a greater or lesser anisotropy, depending on the local degree of alignment of fiber tracts. By taking advantage of the low resistance to fluid flow in white matter, perfusion of large regions of the cerebral hemisphere was possible. Although clinically significant volumes of the brain were perfused with infusate in this study, no attempt was made to perfuse larger brain volumes. Thus, the maximum volume of brain perfusion was not determined and could be much greater than the volumes achieved, because of the continued linear increase in the Vd associated with the increasing Vf (Fig. 2).

Postinfusion Imaging Characteristics. Twenty-four hours after completion of the infusion CT scanning revealed expansion of the Vd, but no further expansion was detected on subsequent imaging (Days 2–5). This behavior is consistent with the diffusional and continued convective spread of iopamidol after the end of infusion (Fig. 4). Because of the ongoing local clearance of iopamidol, the maximal diffusional spread could not be detected, producing an apparent stabilization of the estimated volume of distribution at later observation times (postinfusion Day 2; Fig. 4). Because diffusion and continued convection drive the distribution of an LMW tracer (such as iopamidol) after completion of infusion, the use of imaging to determine the accurate distribution of some LMW molecules may be less accurate at distant time points.

Homogeneity. The distribution of the tracer during infusion was relatively homogeneous in the targeted white matter. An analysis of infusate density on CT scans (Fig. 3) and QAR images (Fig. 6) revealed uniformity and a sharp dropoff at the edges of the infused region during and immediately after infusion. This square-shaped concentration

Discussion

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FIG. 5. Primate 2. Computerized tomography scan (left) and corresponding quantitative autoradiogram (right: 14C-dextran, MW 70 kD) demonstrating the close match between the patterns and regions of distribution on the CT scan and autoradiogram.
Real-time in vivo imaging of CED of an LMW tracer

profile was maintained throughout the period of infusion. These findings are consistent with the bulk-flow properties of CED and the results of a number of previous studies in which convective delivery was used to transport macromolecules (including 14C-albumin) to the CNS. Nevertheless, as expected and consistent with diffusional spread at the perfusion margins after completing infusion, cross-sectional line profiles made through the infused region on postinfusion images (postinfusion Days 1–5) revealed flattening of the concentration distribution pattern and a loss of the square-shaped profile that was observed on real-time CT scanning during infusion (Fig. 3). Despite this flattening on postinfusion images, relatively homogeneous therapeutic levels (that is, levels higher than a desired threshold concentration) of an infused agent could be maintained at distant time points in a targeted region (that is, tissue perfused during convective infusion).

Reliability as a Surrogate Tracer. Iopamidol (777 D) is a reliable surrogate tracer for tracking the Vd during CED of both small (sucrose, 359 D) and large (dextran, 70 kD) molecules characterized by a negligible metabolism and a low microvascular permeation during Vd of approximately 100 µl over infusion times lasting approximately 2 hours. During infusion of a mixture of iopamidol with either sucrose or dextran, the Vd of iopamidol, measured using CT scanning, closely approximated the Vd of both compounds as determined using QAR: the differences were only 19.7 and 7.5%, respectively (Table 2), which correspond to small differences in equivalent spherical radii (difference of 0.3 mm for sucrose and 0.1 mm for dextran). Because the coefficient of variation of Vd for both CT scanning (Table 1) and QAR, given typical radial errors of 7%, is approximately 17%, these differences are not statistically significant. The anatomical location and pattern of distribution of infusate, as determined using CT scanning, corresponded closely with the location and pattern as determined using QAR (Fig. 5).

A critical issue for practical application of these findings is the determination of the range of CED conditions under which iopamidol will accurately trace the spread of the solute and identify when it begins to fail in its role. The use of iopamidol in tracking macroscopic distribution (for example, dextran [MW 70 kD]) is particularly subject to difficulty because the lower molecular weight of iopamidol enhances its spread by diffusional transport and its clearance by microvascular efflux (despite its high hydrophilicity). To quantify this sensitivity, a mathematical model of CED was used to estimate the difference in Vd between iopamidol and dextran over a wide range of concentrations of infusion rates (q) and Vd. These differences were used to estimate the percentage of error in using the Vd of iopamidol to estimate the Vd of dextran.

The CED model was formulated to the first order as an isotropic convection-diffusion-reaction differential mass balance over the infused solute concentration c,

$$\frac{\partial c}{\partial t} = D \nabla^2 c - \frac{1}{\phi} \cdot \nabla \cdot v c - k c,$$

coupled with an inner boundary condition that equates the infused concentration to the interstitial concentration (that is, c_{i} = c (\xi) / \phi where \xi is the catheter radius and \phi is the extravascular volume fraction) and an outer boundary condi-

$$V_{d,\text{mean}} - V_{d,\text{max}} \quad \text{error} = \frac{100.}{}$$

$\text{FIG. 6. Primate 2. Graph showing a line profile of dextran (MW 70 kD) concentration at the end of the infusion, which was derived from QAR of an equatorial tissue section demonstrating a square-shaped distribution pattern indicative of a relatively uniform concentration over the region of infusion and a sharp gradient dropoff at the edges during infusion.}$

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which the absolute error in the estimate of the V_d of dextran age locus corresponds to the set of infusion conditions in and 50%. Thus, the region in Fig. 7 below a given percent-
loci appear in Fig. 7 for the error percentages of 20, 25, and 50%. The shaded region denotes infu-
sion conditions (q_v, V_i combinations) associated with no greater than a 20% error in using the V_i of iopamidol to approximate that of dextran. The circle denotes the q_v–V_i combination used in the ex-
perimental procedure that is described. Note that iopamidol distributions obtained with choices of infusion rates and volumes from the shaded region, but used in conjunction with reactive, partition-
ing, or lipophilic molecules, provide only outer boundary estimates. The equation for the 20% line is V_i (µl) = 600 q_v (µl/minute).

These values were used to determine a q_v–V_i-locus along which the error was maintained at a fixed percentage. These loci appear in Fig. 7 for the error percentages of 20, 25, and 50%. Thus, the region in Fig. 7 below a given percent-
locus corresponds to the set of infusion conditions in which the absolute error in the estimate of the V_i of dextran using iopamidol is less than this percentage. As noted previ-
ously, typical experimental errors in the determination of the V_i of iopamidol alone are approximately 20%. Hence all infusion rate–infusion volume (q_v–V_i) combinations be-
low the 20% line correspond to delivery situations in which errors stemming from iopamidol’s use as a surrogate for a nonreactive macromolecule do not exceed the uncertainty of the measurement itself. Thus, these q_v–V_i combinations are useful for tracking nonreactive compounds using CT scanning with iopamidol, with the caveat that the chosen infusion rate must not be so high that an unacceptable back-
flow along the catheter is encountered. For this reason, infu-
sion rates are often limited to ≤ 0.5 µl/minute for gray matter and ≤ 1 µl/minute for white matter. For q_v–V_i com-
binations above the 20% line (for example, a larger V_i and/ or slower infusion rates), diffusion becomes the dominant force for LMW iopamidol distribution and, as a result, it will no longer have a similar transport behavior (that is, con-
vection) as an HMW molecule such as dextran.

When iopamidol is used to track reactive molecules (in contrast to molecules that are nonreactive and extracellu-
larly localized, such as sucrose and dextran), the same set of q_v–V_i combinations identified earlier may be used. In this case, however, the iopamidol distributions indicate only the boundary of potential tissue penetration because the most reactive compounds would have cleared from the extracell-
ular space well before reaching this boundary. Thus, the boundary of iopamidol serves not only to identify the max-
imum distribution of the pharmacological response in the target region, but also whether sensitive nontargeted regions are likely to lie outside the distribution range of a given drug delivery protocol.

**Safety.** Convective distribution of iopamidol was well tol-
erated and without evidence of a neurological deficit or gross and histopathological evidence of toxicity in rodent and primate models. Although iopamidol is a relatively in-
ert substance, additional studies may be necessary to deter-
mine if an adverse interaction between it and a coinfused therapeutic compound could occur.

**Potential Uses of Surrogate Tracers in CED**

The potential of using CED combined with a surrogate tracer not only to effectively distribute therapeutic mole-
cules to the brain but also to monitor the distribution of infusate noninvasively in real time (including the pattern of distribution, the V_i, and the anatomical location of the infusate) should prove critical for ensuring that the proper distribution of a therapeutic agent needed for maximum efficacy has been achieved. Ideally, the therapeutic comp-
pound and the coinfused tracer would have similar physical and biochemical properties (for example, receptor binding, cellular uptake, metabolic clearance, and the octanol–water coefficient), so that the distribution of the therapeutic comp-
pound could be precisely determined using CT scanning. Consequently, very small (MW < 600 D), reactive, or highly lipophilic compounds, which are not well suited for CED because of rapid efflux across the microvasculature, may not be accurately monitored using this method, unless they are tagged directly with an imaging marker.

Based on the foregoing discussion, as a surrogate tracer certain limitations in the use of iopamidol must be recog-
nized. During CED in the CNS the extracellular fluid of the tissue is being displaced at a rate that depends on the rate of infu-
sion. As long as the displacement of extracellular fluid at the margin of the perfused region is moving radially from the point of infusion at a rate that is faster than the rate of diffusion, distribution by convection will dominate (for drugs with limited degradation in the extracellular fluid, limited binding by the tissue, limited permeation across the vessels and into the circulation, and limited cellular uptake) and produce a distinct boundary of distribution with a sharp drop in concentration at the perfusion boundary during and immediately after infu-
sion. After the infusion is terminat-
ed, however, diffusion and convection continue, albeit at a slower pace, and the slope of the concentration at the mar-
gin of perfusion becomes less steep. Note that in Fig. 7 the maximum infusion volume at which the accuracy of iopam-
idoled for predicting the distribution of an inert drug such as sucrose or dextran has an error no greater than 20% is 800 µl with the peak infusion rate of 1.4 µl/minute; with a V_i/V_i ratio of 5:1 the corresponding volume of tissue perfused at this combination of infusion rate and volume is 4 ml.

**Fig. 7** Graph demonstrating the percentage of error obtained us-
ing the distribution of iopamidol to estimate the V_i of dextran (MW 70 kD) as a function of V_i (in microliters) and volumetric inflow rate (q_v in microliters per minute). Lines denote the loci of constant per-
centages of error of 20, 25, and 50%. The shaded region denotes infu-
sion conditions (q_v, V_i combinations) associated with no greater than a 20% error in using the V_i of iopamidol to approximate that of dextran. The circle denotes the q_v–V_i combination used in the ex-
perimental procedure that is described. Note that iopamidol distributions obtained with choices of infusion rates and volumes from the shaded region, but used in conjunction with reactive, partition-
ing, or lipophilic molecules, provide only outer boundary estimates. The equation for the 20% line is V_i (µl) = 600 q_v (µl/minute).
Real-time in vivo imaging of CED of an LMW tracer

Whether the maximum rate of infusion in humans without backflow along the catheter track will be 1.4 µL/minute has not yet been established and whether the maximal infusion rates in white matter will be comparable remains to be learned; however, even if the maximal infusion rates without backflow in these settings prove to be several-fold greater than 1.4 µL/minute, the Vr for which iopamidol will accurately reflect the Vr of the therapeutic drug will be limited to structures of only a few milliliters in volume. Furthermore, that is the case only for drugs with features of a relatively inert drug, such as described earlier. Thus, iopamidol should be useful for monitoring the distribution of certain LMW drugs in targeted regions of a relatively small volume, regions that might be targets for the treatment of pain, Parkinson disease, tremor, a well-defined epileptic focus, and so forth; but not for disorders requiring higher distribution volumes, such as gliomas and enzymatic storage disorders. Furthermore, the distribution of small, reactive, or lipophilic compounds that do not have features suited to CED may not be accurately monitored by the distribution of iopamidol, even for small volumes.

Conclusions

Real-time in vivo CT scanning of CED in which iopamidol is used as an imaging tracer appears to be safe, feasible, and should be suitable for monitoring the delivery of various LMW and HMW drugs under defined conditions. The application of these findings should enhance the development of novel treatments involving convective delivery of a variety of therapeutic agents.

References


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