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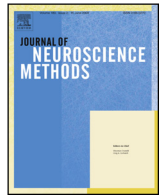

Clinical Neuroscience

Concentration rather than dose defines the local brain toxicity of agents that are effectively distributed by convection-enhanced delivery

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HIGHLIGHTS

- Toxicity of chemotherapeutic agents delivered locally developed in dose- or concentration-dependent manner.
- Toxicities of chemotherapeutic agents with high diffusibility developed in concentration-dependent manner.
- Toxicities of chemotherapeutic agents with limited diffusibility developed in dose-dependent manner.
- Concentration rather than dose affect the local brain toxicity after extensive delivery with convection-enhanced delivery.

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ABSTRACT

Background: Convection-enhanced delivery (CED) has been developed as a potentially effective drug-delivery strategy into the central nervous system. In contrast to systemic intravenous administration, local delivery achieves high concentration and prolonged retention in the local tissue, with increased chance of local toxicity, especially with toxic agents such as chemotherapeutic agents. Therefore, the factors that affect local toxicity should be extensively studied.
New method: With the assumption that concentration-oriented evaluation of toxicity is important for local CED, we evaluated the appearance of local toxicity among different agents after delivery with CED and studied if it is dose dependent or concentration dependent.
Results: Local toxicity profile of chemotherapeutic agents delivered via CED indicates BCNU was dose-dependent, whereas that of ACNU was concentration-dependent. On the other hand, local toxicity for doxorubicin, which is not distributed effectively by CED, was dose-dependent. Local toxicity for PLD, which is extensively distributed by CED, was concentration-dependent.
Comparison with existing method: Traditional evaluation of drug induced toxicity was dose-oriented. This is true for systemic intravascular delivery. However, with local CED, toxicity of several drugs exacerbated in concentration-dependent manner. From our study, local toxicity of drugs that are likely to distribute effectively tended to be concentration-dependent.
Conclusion: Concentration rather than dose may be more important for the toxicity of agents that are effectively distributed by CED. Concentration-oriented evaluation of toxicity is more important for CED.
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1. Introduction

Intravascular injection of agents is immediately followed by dilution and transport away from the injection site to every part of the body by the blood flow. Therefore, the dose of the agent is the basis for the appearance of toxicity, making correct establishment of the maximum tolerable dose important. However, local drug administration may present differences in the toxicity profile. Since local delivery enables high agent concentration at the local infusion site, and prolonged retention of agent in the local tissue, the appearance of toxicity may also depend on the infused concentration of the agent.

Abbreviations: CED, convection-enhanced delivery; BBB, blood–brain barrier; ACNU, nimustine hydrochloride; BCNU, carmustine; PLD, PEGylated liposomal doxorubicin; PBS, phosphate buffered saline; DAPI, 4′,6-diamidino-2-phenylindole; NeuN, neuronal nuclear antigen.

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Convection-enhanced delivery (CED) was developed in the early 1990s as a method for distributing high and low molecular weight compounds directly into the central nervous system by bypassing the blood–brain barrier (BBB), offering an improved volume of distribution compared to simple diffusion (Bobo et al., 1994). CED enables robust distribution of the molecules at the site of infusion, whereas the BBB often hinders the entry of therapeutic agents administered systemically (Buonerba et al., 2011; Groothuis, 2000). Preclinical as well as clinical studies are now actively being carried out to assess the potential as effective treatment for brain tumors (Bidros et al., 2010; Bruce et al., 2011; Kunwar et al., 2010; Lidar et al., 2004; White et al., 2012). During our study to develop CED-based chemotherapy against gliomas, we realized that toxicity profile may show important differences between local CED and systemic administration (Nakamura et al., 2011; Sugiyama et al., 2007, 2012).

The present study demonstrates that concentration rather than dose may affect the local toxicity of chemotherapeutic agents delivered via CED.

2. Materials and methods

2.1. Chemotherapeutic agents

Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea: BCNU) was provided by Ben Venus Laboratories, Inc. (Bedford, OH, USA). To make stock solution, BCNU was dissolved in a 70:30 (v/v) mixture of deionized water and ethanol to a concentration of 50 mg/ml. Infusion solutions of BCNU were prepared by diluting BCNU solution with deionized water. Nimustine hydrochloride (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride: ACNU) was provided by Sankyo Co., Ltd. (Tokyo, Japan). Infusion solutions of ACNU were prepared by diluting ACNU in 0.9% saline to concentrations of 2.0 and 0.5 mg/ml. Doxorubicin was purchased from Sigma–Aldrich (Tokyo, Japan). Stock solutions of free doxorubicin were prepared by diluting doxorubicin in dimethyl sulfoxide to a concentration of 50 mg/ml. Infusion solutions of free doxorubicin were prepared by diluting the stock solution with phosphate buffered saline (PBS). Polyethylene glycol-coated liposomal doxorubicin (PEGylated liposomal doxorubicin: PLD) was purchased from Janssen Pharmaceutical K.K. (Tokyo, Japan). The commercial PLD solutions contained 2 mg/ml of doxorubicin. Infusion solution was prepared by diluting the stock solution with 5% glucose solution.

2.2. Animals

Twelve-week-old male Fischer 344 rats were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan). Protocols used in the animal studies were approved by the Institute for Animal Experimentation of Tohoku University Graduate School of Medicine.

2.3. Convection-enhanced delivery

Infusion was performed using the CED method as described previously (Kikuchi et al., 2008; Saito et al., 2004). Briefly, a reflux-free step-design infusion cannula connected to a 1-ml syringe mounted on a microinfusion pump (BeeHive; Bioanalytical System, West Lafayette, IN, USA) was used to control the infusion rate. Under deep halothane anesthesia, the rats were placed in small-animal stereotactic frames (Narishige Co., Tokyo, Japan). A sagittal incision was made to expose the cranium followed by a burr hole in the skull positioned at 0.5 mm anterior and 3 mm lateral from the bregma using a small dental drill. The following ascending infusion rates were applied to achieve 40 μl total infusion volume: 0.2 μl/min for 15 min, 0.5 μl/min for 10 min, and 0.8 μl/min for 40 min. For 20 μl total infusion volume, the ascending infusion rates were 0.2 μl/min for 15 min, 0.5 μl/min for 10 min, and 0.8 μl/min for 15 min. For 10 μl total infusion volume, the ascending infusion rates were 0.2 μl/min for 15 min, 0.5 μl/min for 10 min, and 0.8 μl/min for 2.5 min.

2.4. Microscopy and image capture

For light microscopy, the sections were imaged with an Eclipse 80i microscope (Nikon, Tokyo, Japan). Images were captured and archived using the Nikon ACT-1 software. For fluorescence microscopy, the sections were imaged with a Dmrxa microscope (Leica Microsystems, Tokyo, Japan) with an excitation wavelength of 561 nm, and images were obtained by a charge-coupled device camera (DFC350 FX; Leica Microsystems). For confocal microscopy, the sections were imaged with a Confocal Microscope C2si (Nikon), and images were captured and archived using the NIS-Elements imaging software (Nikon). Fluorescence generated from doxorubicin and 4′,6-diamidino-2-phenylindole (DAPI) was visualized with excitation wavelengths of 561 nm and 405 nm, respectively.

2.5. Evaluation of toxicity

Healthy male Fisher 344 rats weighing approximately ~250 g were assigned to the following 10 treatment groups: (a) 40 μl CED infusion of BCNU at a concentration of 1.0 mg/ml ($n = 5$); (b) 40 μl CED infusion of BCNU at a concentration of 0.25 mg/ml ($n = 5$); (c) 10 μl CED infusion of BCNU at a concentration of 1.0 mg/ml ($n = 5$); (d) 10 μl CED infusion of ACNU at a concentration of 2.0 mg/ml ($n = 5$); (e) 40 μl CED infusion of ACNU at a concentration of 0.5 mg/ml ($n = 5$); (f) 10 μl CED infusion of doxorubicin at a concentration of 0.4 mg/ml ($n = 5$); (g) 10 μl CED infusion of doxorubicin at a concentration of 0.1 mg/ml ($n = 5$); (h) 40 μl CED infusion of doxorubicin at a concentration of 0.1 mg/ml ($n = 5$); (i) 10 μl CED infusion of PLD at a concentration of 0.8 mg/ml ($n = 10$); and (j) 40 μl CED infusion of PLD at a concentration of 0.2 mg/ml ($n = 10$). Rats were monitored daily for survival and general health, including alertness, grooming, feeding, excreta, skin, fur, mucous membrane conditions, ambulation, breathing, and posture. Animal weights were measured every 3 days. Rats in groups (a)–(h) were deeply anesthetized with diethyl ether, and transcardially perfused with 0.9% saline followed by cold 4% paraformaldehyde on the 15th day after CED treatment. Five rats in groups (i) and (j) were euthanized 15 days after CED, and the other 5 rats in groups (i) and (j) were euthanized 30 days after CED using the same methods. The brains were removed, post-fixed overnight in the same fixative at 4 °C, subjected to paraffin sectioning (4 μm), and histologically examined with hematoxylin and eosin staining and immunohistochemical staining for neuronal nuclear antigen (NeuN). The maximum area of tissues damage was manually delineated from hematoxylin and eosin stained sections. Area of tissue damage was calculated using ImageJ software (V.1.46s, National Institutes of Health). Three independent examiners (R.Z., R.S., Y.M.) performed this calculation and mean value was used for data presentation. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 5 for Windows (GraphPad Software, Inc.; San Diego, CA). The Student's t test was used for two sample comparisons and one-way ANOVA was used for multiple comparisons. Significance was determined at $P < 0.01$.

2.6. Immunohistochemistry

Serial 4-μm thick sections were deparaffinized in three consecutive baths of xylene, followed by rehydration in a graded alcohol series (4 min each in 100%, 95%, and 70% ethanol, and distilled

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