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Original article

Intraperitoneal and intravenous deliveries are not comparable in terms of drug efficacy and cell distribution in neonatal mice with hypoxia–ischemia

Makiko Ohshima [a], Akihiko Taguchi [b], Hidetoshi Tsuda [a], Yoshiaki Sato [c], Kenichi Yamahara [a], Mariko Harada-Shiba [a], Mikiya Miyazato [d], Tomoaki Ikeda [e], Hidehiro Iida [f], Masahiro Tsuji [a,*]

[a] *Department of Regenerative Medicine and Tissue Engineering, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan*
[b] *Department of Regenerative Medicine Research, Institute of Biomedical Research Innovation, Kobe, Hyogo, Japan*
[c] *Division of Neonatology, Center for Maternal–Neonatal Care, Nagoya University Hospital, Nagoya, Aichi, Japan*
[d] *Department of Biochemistry, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan*
[e] *Department of Obstetrics and Gynecology, Mie University School of Medicine, Tsu, Mie, Japan*
[f] *Department of Investigative Radiology, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan*

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Abstract

Background and purpose: Most therapeutic agents are administered intravenously (IV) in clinical settings and intraperitoneally (IP) in preclinical studies with neonatal rodents; however, it remains unclear whether intraperitoneal (IP) injection is truly an acceptable alternative for intravenous (IV) injection in preclinical studies. The objective of our study is to clarify the differences in the therapeutic effects of drugs and in the distribution of infused cells after an IP or IV injection in animals with brain injury.

Methods: Dexamethasone or MK-801, an N-methyl-D-aspartate receptor antagonist was administered either IP or IV in a mouse model of neonatal hypoxic–ischemic encephalopathy. Green fluorescent protein-expressing mesenchymal stem cells (MSCs) or mononuclear cells (MNCs) were injected IP or IV in the mouse model. Two hours and 24 h after the administration of the cells, we investigated the cell distributions by immunohistochemical staining. We also investigated distribution of IV administered MNCs labeled with 2-[^{18}F]fluoro-2-deoxy-D-glucose in a juvenile primate, a macaque with stroke 1 h after the administration.

Results: IP and IV administration of dexamethasone attenuated the brain injury to a similar degree. IP administration of MK-801 attenuated brain injury, whereas IV administration of MK-801 did not. The IV group showed a significantly greater number of infused cells in the lungs and brains in the MSC cohort and in the spleen, liver, and lung in the MNC cohort compared to the IP group. In the macaque, MNCs were detected in the spleen and liver in large amounts, but not in the brain and lungs.

Conclusions: This study demonstrated that the administration route influences the effects of drugs and cell distribution. Therefore, a preclinical study may need to be performed using the optimal administration route used in a clinical setting.

Keywords: Cell transfusion; Dexamethasone; MK-801; Mesenchymal stem cell; Mononuclear cell; Intraperitoneal injection; Intravenous injection; Neonatal hypoxic–ischemic encephalopathy; Primate

* Corresponding author. Address: Department of Regenerative Medicine and Tissue Engineering, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5012; fax: +81 6 6833 9865.
E-mail address: tsuji.masahiro.ri@ncvc.go.jp (M. Tsuji).

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1. Introduction

Children with severe neonatal hypoxic–ischemic encephalopathy (HIE) typically die or develop lifelong neurological impairments [1]. No therapeutic method – except for hypothermia – is available to treat neonatal HIE [2,3]. When treating newborns with HIE in a clinical setting, the administration route for a therapeutic medication is generally intravenous (IV). Postnatal day 7–12 (P7–12) mouse or rat pups are widely used for animal models of neonatal HIE [4]. Although there are several reports on the techniques of IV injection in neonatal rodents [5–7], such studies are difficult to perform accurately because of the small size of the rodent pups. Therefore, most researchers using neonatal rodents choose the intraperitoneal (IP) route as an alternative to the IV route to administer an agent [8–14]. Examining the therapeutic effect of a drug using a administration route other than the expected in clinical settings raises the question whether preclinical evaluations of the agent in neonatal rodents accurately simulate the clinical use of the agent; therefore, investigating whether and how different delivery routes influence the therapeutic effects of agents for neonatal brain injury is very important.

Cell therapies have recently attracted much attention as a novel therapeutic strategy for treating neonatal HIE [15]. Among the several administration methods for cell transfusion, IV administration appears to have the lowest risk for clinical use in HIE. Most studies on cell therapies use IP injection rather than IV injection in neonatal rodents for technical reasons; however, fewer transplanted cells may be distributed in the brain when using the IP route compared to the IV route. When translating neonatal rodent data into clinical trials, the difference between the administration routes is more of a crucial issue for cell therapies than for small chemical compounds.

In this report, we introduce a precise and simple technique of IV injection via the femoral vein in P7–8 mice. The objectives of the study are to clarify whether the IP route is an appropriate administration route for agents in comparison to the IV route. We examined the influence of administration route by injecting the following substances of different sizes: dexamethasone and MK-801 (i.e., small chemical compounds) and mesenchymal stem cells (MSCs) and mononuclear cells (MNCs) (i.e., large substances) in a mouse model of neonatal HIE. Dexamethasone is a steroid hormone, has anti-inflammatory effects, and exerts neuroprotective effects against hypoxic–ischemic (HI) brain damage [16–18]. MK-801 is an N-methyl-D-aspartate (NMDA) receptor antagonist and exerts neuroprotective effects by blocking NMDA type glutamate receptors expressed in neurons [19,20]. MSCs are adhesive cells derived from culturing mesenchymal tissue such as bone marrow and adipose tissue and have the potential to differentiate into several cell types such as muscle and bone [21–24]. The MNC fraction of bone marrow contains a variety of blood cells including hematopoietic stem cells [25]. Furthermore, to clarify whether different recipient animals show different distributions of infused cells, we examined the systemic distribution of intravenously (IV) transfused cells in a non-human primate, a macaque with ischemic brain injury.

2. Materials and methods

All experiments were performed in accordance with protocols approved by the Experimental Animal Care and Use Committee of the National Cerebral and Cardiovascular Center.

2.1. Hypoxia–ischemia procedure

HI was induced in eight-days-old (i.e., P8) CB17 mouse pups (CLEA Japan Inc., Tokyo, Japan) as previously described [26]. In brief, P8 CB17 mouse pups (with a body weight of 4.5 ± 0.1 g) were anesthetized with isoflurane. The left carotid artery was permanently occluded, and after a one-hour recovery period, the pups were subjected to hypoxia (8% oxygen) for 30 min.

2.2. IV injection via the femoral vein

The materials included a 35 G needle (ReactSystem, Osaka, Japan), which has an outer diameter of 0.15 mm and an inner diameter of 0.1 mm, a 100-μl Hamilton syringe, scissors, and forceps. Each mouse pup was anesthetized and was laid on its back. The limbs were immobilized with tape pasted to an operating board. The skin over the left femoral vein was incised from the inguinal region to 5 mm distal from the incision. The adipose tissue over the vessel was removed, and the femoral vein was exposed. Using the 35 G needle, we manually injected solutions under a stereoscopic microscope (Fig. 1). Intravascular administration was easily confirmed by observing the infused solution, which was transparent fluid in the red blood, flowing from the tip of the needle into the bloodstream. Extremely slow withdrawal of the needle caused no bleeding in approximately 30% of the pups. To stop the bleeding after needle withdrawal, a cotton swab was pressed onto the injection site immediately after pulling the needle from the vein, and ligating the vessel was unnecessary.

2.3. Drug administration

We used dexamethasone and MK-801, which are neuroprotectants [19,20,27,28]. The mouse pups were randomly assigned to one of three groups in each drug cohort. *The dexamethasone cohort*: according to reports showing its neuroprotective effects [16,26], dexamethasone