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A NEW APPROACH WITH LESS DAMAGE: INTRANASAL DELIVERY OF TETRACYCLINE-INDUCIBLE REPLICATION-DEFECTIVE HERPES SIMPLEX VIRUS TYPE-1 VECTOR TO BRAIN

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Abstract—Gene therapy holds great potential for treating neurological disorders. However, delivering gene vectors to the brain has been either invasive or inefficacious in most studies to date. The aim of this study was to develop a safe and efficacious strategy for delivering gene vectors to the brain. A tetracycline-inducible replication-defective herpes simplex virus type-1 vector, QR9TO-LacZ, was administered to rats intranasally. QR9TO-LacZ could infect primary cortical neurons and express the reporter gene without detectable replication. QR9TO-LacZ was observed in the olfactory bulb, hippocampus, striatum, cortex, medulla, cerebellum, ventricles, and nasal septum after intranasal administration. Expression of the reporter gene could be controlled effectively by tetracycline. *In vitro*, introduction of QR9TO-LacZ did not change the structure of transfected neurons. *In vivo*, QR9TO-LacZ did not increase apoptosis in neurons and did not alter levels of interleukin 6 and tumor necrosis factor α in the brain after intranasal delivery. Our data suggest that intranasally applied QR9TO-LacZ has a wide distribution and expresses the reporter gene in the brain under the control of tetracycline with less cytotoxicity than intravenous or stereotactic delivery methods.

Key words: intranasal, HSV-1, brain, trigeminal, vector, tetracycline.

Tremendous progress has been made in gene therapy, and great success has been achieved in its clinical application, despite years of skepticism from the scientific community and neglect by the pharmaceutical industry (Aiuti et al., 2009; Cartier et al., 2009). Viral vectors are the most frequently used vectors for gene therapy. Herpes simplex virus type-1 (HSV-1), a neurotropic virus, displays several important adaptations, which can be rationally exploited in the design of gene therapy vectors for neurological applications (Frampton et al., 2005; Manservigi et al., 2010). However, a common concern with the use of viral vectors is whether they can be delivered to target tissues in a sensitive and tightly regulated manner (Stieger et al., 2009). QR9TO-LacZ, a tetracycline-inducible replication-defective HSV-1 vector, employs the tetracycline-regulated mammalian expression system and an HSV-1 recombinant K0R. The K0R uses two copies of the tetracycline repressor (tetR) gene to replace the infected cell polypeptide 0 (ICP0) gene and encodes tetR under the control of the ICP0 promoter. The tetR acts as a potent repressor that down-regulates gene expression when tetracycline is absent. This specific design allows an early and high level of expression of tetR upon virus entering the cell (Yao and Eriksson, 1999). Currently, the routes used for vector delivery to the brain are either invasive, such as intracerebroventricular injection, or inefficacious as a result of the intact blood–brain barrier (BBB), such as intravenous injection (Neuwelt et al., 2011). Thus, developing a safe and efficacious approach for gene vector delivery to the brain is essential for the use of gene therapy to treat CNS disorders.

Intranasal delivery is a novel therapeutic delivery method that is noninvasive, essentially painless, and easily administered by the patient or a physician (Dhuria et al., 2010). It has been shown to be effective at delivering large molecular-weight peptides, hydrophilic chemical drugs, metals, plasmids, bacterial phages, and stem cells that cannot easily cross the BBB into the brain directly and quickly (Perl and Good, 1987; Frenkel and Solomon, 2002; Han et al., 2007; Bitko and Barik, 2008; Hashizume et al., 2008; Alcalá-Barraza et al., 2010; Danielyan et al., 2011; Jiang et al., 2011b). Intranasal delivery of drugs could be used to protect neurons against sorts of insults, including ischemic stroke and Alzheimer's disease (De Rosa et al., 2005; Jiang et al., 2011a). Substances gain access to the brain following intranasal administration through olfactory, trigeminal, vascular, or cervical node routes (Dhuria et al., 2010). Previous studies have demonstrated that many viral vectors, such as the HSV-1 vector, can be targeted to the olfactory bulb and/or the brain by intranasal delivery (Holtmaat et al., 1996; Jerusalmi et al., 2003; Doi et al., 2005; Laing et al., 2006).

However, replication of wild HSV-1 occurred in the trigeminal ganglia and to a lower extent in the brain 4 and

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Abbreviations: BBB, blood–brain barrier; DAB, diaminobenzidine; ELISA, enzyme-linked immunosorbent assay; HSV-1, herpes simplex virus type-1; ICP0, infected cell polypeptide 0; IL6, interleukin 6; m.o.i., multiplicity of infection; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; PBS, phosphate buffer saline; PFU, plaque-forming units; POD, peroxidease; tetR, tetracycline repressor; TNF-α, tumor necrosis factor α; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling assay; X-gal, 5-bromo-4-chloro-3-indolyl beta-galactoside; β-Gal, β-galactosidase.

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7 days postinfection following intranasal delivery (Broberg et al., 2004). In addition, intranasal inoculation of a replication-defective recombinant adenoviral vector led to little or no viral dissemination to other regions of the brain even when there was a moderate level of gene transfer to the olfactory bulb (Damjanovic et al., 2008). Furthermore, limited research is available on the pathway by which the HSV-1 vector travels from the nose to the brain. In the present study, we aimed to determine the safety and efficacy of the tetracycline-inducible replication-defective HSV-1 vector QR9TO-LacZ in delivering a reporter gene to the CNS and expressing this gene under the control of tetracycline after intranasal administration.

EXPERIMENTAL PROCEDURES

Animals

All procedures were performed under the guidelines published in the NIH guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996) and approved by the Animal Care Committee (Institute of Science and Technology, Jiangsu Province, China). Adult male Sprague–Dawley rats (220–250 g) were provided by the Model Animal Research Center of Jinling Hospital (Nanjing, Jiangsu, China). The rats were housed under controlled environmental conditions with an ambient temperature of 25 °C, relative humidity of 65%, and 12/12-h light–dark cycles. Food and water were provided *ad libitum*. All efforts were made to minimize the number of animals used and their suffering.

Cells

RUL9-8 cells (provided by Dr. Yao, Harvard, Boston, MA, USA) (Yao et al., 1998), a double-stable cell line expressing both tetR and UL9 that was established by stable transfection of U2CEP4R11 cells with pcDNA-UL9, were grown and maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 50 μg/ml Hygromycin B (Invitrogen), and 100 mg/ml G418 (Invitrogen). RUL9-8 cells can efficiently support the growth of an HSV-1 UL9 insertion mutant. Primary cortical neurons were prepared from newborn Sprague–Dawley rats as described previously (Chen et al., 2010). After trituration and trypsinization, the single-cell suspension was seeded unto 6-well or 96-well plates coated with poly-L-lysine (Sigma, St. Louis, USA) and incubated for 10 days, the time required for maturation of cortical neurons.

Vector

A tetracycline-inducible replication-defective HSV-1 strain (QR9TO-LacZ, provided by Dr. Yao) (Yao et al., 2006) was propagated, and plaque assays were performed in RUL9-8 cells. QR9TO-LacZ is a replication-impaired virus in which the HSV-1 ICP0 gene form the KOS strain is knocked-down, and the essential UL9 gene is replaced with the LacZ gene driven by the tetracycline operator sequence-containing hCMV major immediate-early promoter.

Infection of QR9TO-LacZ

Neurons and RUL9-8 cells were seeded at a density of approximately 1×10^6 cells per 60-mm dish. Ten days or 48 h after seeding of neurons and RUL9-8 cells, respectively, the cells were either mock infected or infected with QR9TO-lacZ. Cells were infected in the absence or presence of tetracycline, as described in the text.

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

An MTT assay was used to determine the viability of neurons after 7 days of infection with QR9TO-LacZ with an m.o.i. of 0, 3, 5, 10, and 30 plaque-forming units (PFU)/cell. Neurons were incubated for 60 min with 500 μg/ml MTT reagent (Sigma) in the dark. After incubation, medium was removed, and the Formazan dye was extracted using 100% ethanol. The absorbance at 590 nm was determined using a microplate reader (Bio-Rad, California, USA).

Detection of β-Gal reporter gene activity

RUL9-8 cell extracts were used to test the β-galactosidase (β-Gal) activity using a β-galactosidase reporter gene activity detection kit (Sigma) 72 h postinfection. Fifty microliters of cell extract and 50 μl of working solution were pipetted into each well of a microplate and incubated for 30 min at room temperature. After adding 150 μl of stop solution to each well, the microplate was read at 420 nm using a microplate reader system (Bio-Rad).

Vector delivery

Rats were anesthetized with 40 mg/kg pentobarbital sodium i.p. (Sigma) 1 week after feeding with tetracycline. For intranasal administration of the vector, it was performed as previously described with minimal modifications (Jiang et al., 2011a). The anesthetized rats were placed in a supine position, and the head and neck were maintained horizontal with a small roll of gauze under the dorsal neck. Approximately 40 μl of QR9TO-LacZ at a dose of 10^8 PFU was administered by pipetting 2 μl drops, alternating between each naris every 2 min, for a total of 40 min. During administration of each drop, to facilitate snorting of the drops high into the nasal cavity, the contralateral naris was occluded gently. For intracerebral delivery of vectors, rats were placed in a stereotaxic frame. Stereotaxic injections were made by Hamilton syringe using the following coordinates: 0.5 mm rostral to bregma, 3.5 mm lateral to midline, and 5.5 mm ventral to the skull surface. Rats were given 10 μl of vector at a dose of 10^8 PFU. After injection, the needle was left in place for 5 min before being slowly withdrawn. For intravenous delivery of vectors, rats were given 40 μl of vector at a dose of 10^8 PFU. After administration of the vector, the rats were given tetracycline-containing water.

Trigeminal nerve ectomy

To determine the pathway of vector migration following intranasal delivery, the first branch of the trigeminal nerve (V1) was removed before intranasal administration of the vector because the V1 is located in the upper part of nasal cavity (Tucker, 1971). After anesthetization, rats were fixed in a stereotaxic frame. An incision was first made along the superior orbital fissure, and then the eyeball was gently pulled aside to expose the V1. The V1 was cut by electrocoagulation, and finally, the wound was sutured. After the operation, intranasal delivery of the vector was carried out as described in the vector delivery section.

Electron microscopy

After 72 h of infection, cells were fixed (2.5% glutaraldehyde in phosphate buffer saline, PBS, pH 7.4) for 1 h at 4 °C and then postfixed in 1% OsO_4 in cacodylate buffer (0.12 M, pH 7.4) for 45–60 min, dehydrated, and embedded in Epoxy resin. Ultramicrotome (Ultracut E, Reichert-Jung, New York, USA) 50 nm sections were then examined by a Philips CM 10 transmission electron microscope (Netherland). Images were taken with a Mega View II digital camera (Soft Imaging System).