Journal of Neuroscience Methods 219 (2013) 271–275

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

Superparamagnetic beads for estimation of spinal subarachnoid space permeability in rats

Rebecca E. Franco-Bourland [a,*], Gabriel Guízar-Sahagún [b,c], Alejandra Quintana-Armenta [a], Horacio José Reyes-Alva [c,d], Angelina Martínez-Cruz [c], Ignacio Madrazo [b,c]

[a] Department of Biochemistry, Instituto Nacional de Rehabilitación, Calzada Mexico-Xochimilco 289, 14389 Mexico City, Mexico
[b] Research Unit for Neurological Diseases, Instituto Mexicano del Seguro Social, Tlalpan 4430, 14050 Mexico City, Mexico
[c] Department of Experimental Surgery, Proyecto Camina A.C., Tlalpan 4430, 14050 Mexico City, Mexico
[d] Department of Neurology, School of Veterinary Medicine, UAEM, Mariano Matamoros Sur 706, 50130 Toluca, Mexico

HIGHLIGHTS

- Spinal subarachnoid space patency is essential for spinal cord and root integrity.
- Subarachnoid permeability is estimated by passage of superparamagnetic beads in CSF.
- The method is highly reproducible and sensitive to detect low subarachnoid patency.
- This method may be useful to grade spinal subarachnoid obstruction in the rat.

ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 24 July 2013
Accepted 5 August 2013

Keywords:
Cerebrospinal fluid flow
Intrathecal
Laminectomy
Obstruction
Tracer
Blood contamination
Colorimetric measurement

ABSTRACT

Background: Human spinal pathological processes have been linked to a loss of spinal subarachnoid space (SSAS) permeability, which has therefore become a target for therapy. Hence, it has become important to measure SSAS patency in rat models of these human disorders.
New method: The estimation of *in vivo* rat SSAS patency is described by quantifying passage of streptavidin-covered superparamagnetic beads (SPMB) in cerebrospinal fluid (CSF). Beads are injected into the *cisterna magna* and recovered at spinal level L2. They are then coated with biotynilated horseradish peroxidase for enzymatically based colorimetric measurement, after removal of bloody CSF to avoid interference with the colorimetric readings. The procedure was tested in intact rats and in rats 24 h after T9 laminectomy. Residual beads in SSAS were viewed by histology.
Results: Average bead recovery from intact rats was 6.4% of amount initially administered, in a mean CSF volume of 126 μL; in laminectomized rats, it was 1%, in a mean CSF volume of 39.2 μL.
Comparison with existing method(s): Unlike *in vivo* imaging techniques, such as myelography (used here to validate our method) and near infrared fluorescence technology for qualitative rat SSAS patency viewing, our SPMB-based method allows for an *in vivo* quantitative estimation of the permeability of this space.
Conclusions: A novel method has been established to reliably determine SSAS permeability in rats. The method is reproducible and has the required sensitivity to detect an 84.4% reduction in bead recovery, as seen in laminectomized rats compared to intact animals.

1. Introduction

The subarachnoid space is the gap between the arachnoid membrane and *pia mater*, in which the cerebrospinal fluid (CSF) is contained. Subarachnoid space permeability is essential for CSF to reach the central nervous system ensuring its integrity (Brodbelt and Stoodley, 2007; Yoshizawa, 2002; Zappaterra and Lehtinen, 2012). Spinal subarachnoid space (SSAS) is the major route of CSF flow for spinal cord and nerve roots. Partial or complete reduction of SSAS can result from several spinal diseases as well as traumatic spinal cord injury (Brodbelt and Stoodley, 2007; Caplan et al., 1990; Grossman and Krabak, 1999; Reyes-Alva et al., 2013). SSAS blockage could be involved in the pathophysiological mechanisms underlying these disorders and therefore could be a target for therapy (Maxmauer et al., 2011). Consequently, it is important to be able to measure SSAS permeability in rat models of these human disorders.

Abbreviations: SSAS, spinal subarachnoid space; CSF, cerebrospinal fluid; SPMB, superparamagnetic beads; CM, cisterna magna; TMB, tetramethylbenzidine.

* Corresponding author. Tel.: +52 55 5999 1000x19508; fax: +52 55 5573 5545.
E-mail addresses: refrancobourland@gmail.com, rfranco@inr.gob.mx (R.E. Franco-Bourland).

0165-0270/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneumeth.2013.08.004

The simplest and most accessible procedure to evaluate rat SSAS permeability is by injecting a dye into SSAS and recovering it beyond the suspected site of blockage. To estimate SSAS permeability in live rats, we originally tried the use of riboflavin and indocyanine green as quantitative colorimetric and fluorescent CSF tracers, respectively. However, hematogenous contamination of CSF samples prevented reliable measurements.

The present study was therefore designed to test the hypothesis that rat SSAS patency could be estimated *in vivo* by the quantitative passage of superparamagnetic beads (SPMB) as tracer in the CSF, even in the presence of blood contamination. Accordingly, our objective was to establish a surgical procedure to inject the beads in the *cisterna magna* (CM), recover them at L2, rid them of contaminating blood for final measurement in an enzyme-linked colorimetric assay, in both intact and laminectomized rats.

2. Materials and methods

2.1. Experimental design

A method was developed to determine SSAS permeability using SPMB in live rats that involved the following steps: first, optimization of the enzymatic assay used to quantify the beads; secondly, development of the surgical procedure for *in vivo* bead injection and recovery, and finally optimization of the amount of beads injected. After refinement, the method was tested in a group of intact rats ($n = 8$), and then in a group of rats laminectomized at T9 24 h before the procedure ($n = 6$). Variables measured were: recovered SPMB (μg) and CSF (μL). The method was validated by myelography using both control and laminectomized rats ($n = 3$). Histology was performed to view SSAS ($n = 2$).

2.2. Animals and materials

Animal procedures were approved by our animal ethics committee and carried out in accordance to Mexican law (NOM-062-ZOO-1999) and NIH guidelines for animal care. All procedures were carried out in adult female Long–Evans rats, weighing 240–260 g.

Superparamagnetic beads (Dynabeads MyOne Streptavidin T1, 1 μm in diameter with a monolayer of streptavidin covalently coupled to the surface), the magnet to separate the beads (DynaMag™-Spin), and biotinylated horseradish peroxidase (catalog # 43-2040) were from Invitrogen. Tetramethylbenzidine (TMB)-hydrogen peroxide solution and diluted H_2SO_4-based stop solution were from the ELISA rat tumor necrosis factor α kit (Biosource).

2.3. Surgical procedures and bead delivery-recovery protocol

Surgical procedures were performed under ketamine/xylazine anesthesia as previously described (Reyes-Alva et al., 2013). For bead delivery in the CM, a laminectomy was performed at C1/C2 and at L2 for bead recovery. Beads (200 μg in 20 μL of the original undiluted bead suspension) were injected in the CM after removing an equal volume of CSF, using a 30-gauge needle with the bevel facing caudally, attached to a 50 μL-Hamilton microsyringe (the needle was left in place until CSF was distally recovered to prevent bead leakage). Immediately after bead administration, the dural sac exposed at L2 was torn dorsally with a microknife and all CSF (usually bloody) was recovered using heparinized capillaries, transferred to Eppendorf tubes, and finally total CSF volume was measured (approximately 80–160 μL for intact rats). For bead delivery and recovery, animals were placed on a 45° inclined plane in the cephalic-caudal direction. Procedures were carried out under microscopic viewing.

Once this procedure was standardized in intact rats, it was further tested in a group of rats subjected to laminectomy at T9, 24 h before bead delivery.

2.4. Bead handling and measurement

Initially CSF-suspended beads were subjected to 4 cycles of magnetic separation/washing in 0.01 M phosphate buffered saline, pH 7.2 (PBS); they were then mixed for 30 min in a diluted biotinylated peroxidase solution in PBS containing 0.1% bovine serum albumin (BSA) (0.2 mg/mL). To ensure complete removal of excess enzyme, enzyme-coated beads were magnetized/washed 3 times using 125 μL of the PBS/BSA diluting buffer. Beads were then incubated for 5 min in 100 μL of the original TMB/hydrogen peroxide solution and the reaction stopped by adding 100 μL of the original undiluted H_2SO_4-based stop solution. Within the next 30 min 100 μL of the reaction mixture were placed in wells of a polystyrene 96-sample plate and read at 450 nm in a Biorad Microplate Reader, model 450. Color was directly proportional to the amount of bead-bound enzyme, which in turn was proportional to the amount of beads recovered. UV measurements were read off calibration curves.

2.5. Myelography

Myelography was performed as previously described (Reyes-Alva et al., 2013). Minor modifications included the use of Optiray™ 300 as contrast medium and the CMR-X Ray MRH II E Series generator platform.

2.6. Histology

Deeply anesthetized control and laminectomized rats were perfused with buffered 10% formaline. Conserving meninges, spinal cord blocks from the site of laminectomy and one just above it were embedded in paraffin. Five micron-thick transverse sections were stained with hematoxylin and eosin. Due to the small size of the SPMB (1 μm in diameter), microscopic observations were made using a 100× objective.

2.7. Statistical analysis

Comparison of bead and CSF volume recovery between intact and laminectomized animals were performed using unpaired Student's *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

SSAS patency in intact rats allowed for the recovery of 12.84 μg of beads on average, which was 6.4% of the 200 μg administered initially (Fig. 1A). Mean CSF volume recovered from these rats was 126.0 μL (Fig. 1B).

In laminectomized rats, 24 h after surgery, the average bead recovery was 1.99 μg, that is 1% of the amount of beads administered initially, or 15.6% compared to beads recovered in intact rats ($p < 0.001$, Fig. 1A); CSF recovered was on the average 39.2 μL, 31.1% compared to that recovered in intact rats ($p < 0.01$, Fig. 1B).

The method as a whole was reproducible and with the required sensitivity to enable us to detect the significantly reduced SSAS permeability in laminectomized rats as compared to intact animals.

The method was validated by myelography. Lateral myelograms in all intact controls showed the permeability of the subarachnoid space judging from the presence of the dorsal contrast medium column in the lumbo-sacral region. In 2 of the 3 laminectomized rats contrast medium could not be seen beyond the site of injury,