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Comparing levels of biochemical markers in CSF from cannulated and non-cannulated rats

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ABSTRACT

Cerebrospinal fluid (CSF) is commonly used for assessing biomarkers of drug efficacy or disease progression in the central nervous system. Studies of CSF from pre-clinical species can characterize biomarkers for use in clinical trials. However, obtaining CSF from pre-clinical species, particularly rodents, can be challenging due to small body sizes, and consequently, low volumes of CSF. Surgical cannulation of rats is commonly used to allow for CSF withdrawal from the cisterna magna. However, cannulae do not remain patent over multiple days, making chronic studies on the same rats difficult. Moreover, CSF biomarkers may be affected by cannulation. Thus cannulation may contribute confounding factors to the understanding of CSF biomarkers. To determine the potential impact on biomarkers, CSF was analyzed from cannulated rats, surgically implanted with catheters as well as from non-cannulated rats. Brain protein biomarkers (αII-spectrin SBDP150 and total tau) and albumin, were measured in the CSF using ELISA assays. Overall, cannulated rat CSF had elevated levels of the biomarkers examined compared to non-cannulated rat CSF. Additionally, the variation in biomarker levels observed among CSF from cannulated rats was greater than that observed for non-cannulated rat CSF. These results demonstrate that in some cases, biomarker assessment using CSF from cannulated rats may differ from that of non-cannulated animals and may contribute confounding factors to biomarker measurements and assay development for clinical use.

1. Introduction

Efforts to characterize disorders of the central nervous system or to discover therapeutics thereof commonly rely on analysis of cerebrospinal fluid (CSF) for indicators of disease, disease progression, pre-clinical model utility, and drug distribution or efficacy. CSF is in direct contact with the brain and thus is an ideal fluid for assessing changes in the brain. Sampling CSF from human subjects is typically performed via lumbar puncture or indwelling lumbar catheter, and can yield multiple, high volume (milliliters), blood-free samples each day. CSF sampling from laboratory animals is more of a challenge due mostly to their small body size. In recent reports in which rat models are used for CNS research, two main techniques for collecting CSF are employed. Some investigators (Wang et al., 2004; El Mouedden et al., 2005; Hoistad et al., 2005) use rats having surgically implanted cannulas that access the cisterna magna while others (Takasugi et al., 2005; Best et al., 2006; Sharma et al., 2006) utilize a technique recently detailed (Nirogi et al., 2009) whereby a small 'butterfly' needle is guided, transcutaneously, into the cisterna magna at the time of collection. No other incision or surgical technique is employed in the latter method.

We were interested in comparing the practical uses of these two collection methods as well as the biochemical composition of CSF obtained by each. To this end, cannulated and normal Sprague–Dawley rats were employed to provide CSF via the cisterna magna. In all, 365 CSF samples were collected from 144 rats; 71 of the rats were cannulated and 73 were non-cannulated. Levels of protein biomarkers indicative of blood–brain barrier integrity (albumin) or neuronal damage (t-tau and SBDP150) were measured to shed light on the two methods of CSF collection. The permeabil-

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ity of rat albumin into CSF is well characterized (Habgood et al., 1992) and elevated albumin in the CSF of an adult rat can indicate a compromise in the blood–brain barrier (Westergren and Johansson, 1991). As CSF total tau (t-tau) is neuronally derived, elevation of t-tau immunoreactivity above normal levels in CSF has been shown to indicate neuronal damage (Ost et al., 2006; Palmio et al., 2009). Finally, SBDP150 is a 150 kDa breakdown product of non-erythroid αII-spectrin (SBDP150) driven by the cysteine protease calpain (Wang et al., 1998; Wang, 2000; Glantz et al., 2007). Elevation of this biomarker in the CSF can indicate neuronal death by necrosis resulting from brain injury (Pike et al., 2001; Pineda et al., 2007; Zhang et al., 2009).

2. Methods

2.1. Animals

Adult Sprague–Dawley rats (male, weighing 200–400 g), obtained from Charles River Laboratories International, Inc. (Wilmington, MA) were used for all experiments. All animals were housed at Abbott Laboratories in a temperature-regulated environment under a controlled 12-h light/dark cycle, with lights on at 6:00 AM. Food and water were available *ad libitum* at all times except during testing. Abbott's animal facilities are approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All testing was done after procedures outlined in protocols approved by Abbott's Institutional Animal Care and Use Committee.

2.2. Intracisternal cannulation

Rat intracisternal cannulation was performed at Charles River Laboratories (Web reference) using aseptic technique; no pre- or post-surgery antibiotics were utilized. In summary, the anesthetized rat was mounted onto a stereotaxic device and a midline incision was made on top of the head to expose the parietal and inter-parietal areas of the skull. Holes were drilled at the parietal region and mounting screws were secured in the holes. A hole was then drilled at the external occipital crest. A guide cannula was inserted through the hole on the occipital crest and advanced to the cisterna magna. Cranio-plastic powder was applied around the cannula, which served to affix the cannula and to cover the exposed portion of the skull. A small amount of cranio-plastic liquid was applied to the powder. Finally, a small length (3–4 mm) of polyethylene tubing (I.D. 0.58 mm, O.D. 0.965 mm) was wrapped around the protruding end of the cannula and sealed with heated forceps, forming a plug to prevent CSF leakage.

2.3. General CSF collection procedures

Prior to CSF collection, the rats were anesthetized in an induction chamber with inhaled isoflurane (3–4% mixed with oxygen). During CSF collection, a nose-cone was used for delivering the isoflurane. The volume of CSF collected was between 50 and 100 μl regardless of collection method. Only clear CSF samples with no sign of blood contamination or discoloration were included in this study. After collection, the CSF sample was centrifuged at 2000 × *g* for 3 min, transferred to a new siliconized polypropylene vial, and frozen at −80 °C until testing. When more than one sample was taken per rat, the samples were taken at least 6 h apart.

2.3.1. Cannulated rat CSF collection

For the cannulated rats, the CSF was collected 2–4 days after surgery. This timeframe proved optimal for successful sampling as later attempts were much less successful due to loss of patency. Briefly, with the rat on its side, the polyethylene plug is removed from the cannula allowing CSF to flow into a siliconized polypropylene vial.

2.3.2. Non-cannulated rat CSF collection

For the non-cannulated rats, the fur was shaved from above the occipital crest and down to the base of the neck. This area was then aseptically prepped. Positioning the head at approximately a 45° downward angle, thus forming a depressible surface behind the occipital crest, a 25 gauge × 3/4 in. needle attached to polyethylene tubing (Butterfly Infusion Set, Abbott Laboratories, North Chicago, IL) is slowly inserted, vertically, into the depressible region. Once the needle breaches the dura above the cisterna magna, CSF flows into the needle and becomes visible in the tubing. Caution is taken not to move the needle until the complete sample has been collected. The CSF is then expelled into a siliconized polypropylene vial using a syringe.

2.4. Biomarker quantification by ELISA

Biomarker quantification was performed using ELISA kits as described below. Reagents from identical lots were used for assessing biomarker levels in cannulated and non-cannulated rat CSF. For each ELISA, the average coefficient of variation in signal from calibrators in the standard curve was less than 20% across plates, demonstrating good plate to plate reproducibility and allowing for reliable comparison of biomarker concentrations derived from different plates.

Due to CSF volume restrictions, not all three biomarkers were measured from each CSF sample. From 21 cannulated and 135 non-cannulated rats, albumin and SBDP150 were measured from the same CSF sample. The CSF samples used for t-tau concentrations were not analyzed for either of the other two biomarkers.

2.4.1. Albumin quantification in CSF

Albumin was measured using Rat Albumin ELISA kit from Immunology Consultants Lab, Inc. (Newburg, OR) as per manufacturers protocol. The CSF was diluted serially in the ELISA diluent 1:10,000 and 100 μl of the diluted sample was used per well of the micro-titer plate. Signal for each sample was above the lower limit of quantification.

2.4.2. SBDP150 quantification in CSF

The assay for αII-spectrin breakdown product (SBDP150) was developed in-house using the electrochemiluminescence detection technology from Meso Scale Discovery (MSD) (Gaithersburg, Maryland). The following assay-specific parameters were validated for optimal SBDP150 detection in CSF. Each well of an MSD bare high bind plate was coated with 50 ng/well using 25 μl of a 2 μg/ml solution of goat anti-αII-spectrin-BDP150 kDa (SDBP150 antibody was made in-house at Banyan Biomarkers, Inc.); the antibody dilution was prepared in phosphate buffered saline (PBS) and the coating step was conducted at room temperature overnight. After coating, the plate was blocked with 150 μl of 3% MSD Blocker A in PBS containing 0.05% Tween 20 (PBST) with vigorous shaking (300–1000 rpm) for 1 h. After washing 3× with PBST, 25 μl of CSF or calibrator standards were added per well and incubated at 4 °C with vigorous shaking over night. For this step, the calibrator was diluted in 1% MSD Blocker A in PBST while the CSF samples were undiluted. The next day the plate was washed 3× with PBST and incubated with 25 μl/well of mouse anti-αII-spectrin antibody (MAB1622 from Millipore, Billerica, MA) (1 μg/ml in 1% MSD Blocker A in PBST). This incubation took place at room temperature with vigorous shaking for 2 h. The plate was washed 3× with PBST and 25 μl/well of anti-mouse SULFO-TAG™ labeled detection antibody (1 μg/ml in 1% MSD Blocker