Journal of Neuroscience Methods 205 (2012) 36–44

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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

Basic Neuroscience

Cisterna magna cannulated repeated CSF sampling rat model – effects of a gamma-secretase inhibitor on Aβ levels

Jennifer S. Shapiro*,[3], Mark Stiteler[3], Guoxin Wu, Eric A. Price, Adam J. Simon[1], Sethu Sankaranarayanan[2]

Merck & Co., Inc., Neuroscience, 770 Sumneytown Pike, PO Box 4, West Point, PA 19486, USA

ARTICLE INFO

Article history:
Received 24 August 2011
Received in revised form
19 December 2011
Accepted 20 December 2011

Keywords:
Cerebrospinal fluid
Cisterna magna cannulation
Repeated CSF sampling
Alzheimer's
gamma-secretase
Amyloid beta
Abeta

ABSTRACT

Cerebrospinal fluid (CSF) provides a window into central nervous system (CNS) physiology and pathophysiology in human neurodegenerative conditions such as Alzheimer's disease. Changes in CSF bioanalytes also provide a direct readout of target engagement in the CNS following pharmacological interventions in clinical trials. Given the importance of tracking CNS bioanalytes in drug discovery, we have developed a novel cisterna magna cannulated rat model for repeated CSF sampling and used it to assess an amyloid beta (Aβ) lowering agent. The surgically implanted cisterna magna cannula was patent over a period of 1–2 weeks and enabled repeated sampling of CSF (volume of ~30–50 μL/sample) from each rat. CSF Aβ40 levels showed good intra-animal variability across time points and inter-animal variability within a time point. Peripheral treatment with a gamma-secretase inhibitor (GSI) led to a rapid and robust decline in CSF Aβ40 levels that returned to baseline over 24–96 h after dosing. Terminal brain, CSF and plasma Aβ levels measured at 24 h after dosing demonstrated robust Aβ lowering and showed excellent correlation across these compartments. These results are the first pharmacological validation of the repeated CSF sampling rat model for Aβ lowering agents. This model can have broad applicability in pharmacological evaluation for diverse CNS targets.

1. Introduction

Human CSF, which is derived from the chorionic villi lining the brain ventricles, is in communication with the brain extracellular space and enables tracking of CNS biochemical changes and pharmacokinetics of peripherally administered brain-penetrant compounds over time (Shen et al., 2004; Lin, 2008). CSF sampling has enabled evaluation of biochemical changes in CNS conditions such as brain injury, stroke, post-surgical recovery (Kandiah et al., 2006; Petzold, 2007; Riou et al., 2008; Maurer, 2010) and different neurodegenerative conditions (Hu et al., 2010; van Dijk et al., 2010). Numerous studies have demonstrated a decline in CSF Aβ42 levels and modest elevation of CSF total-Tau and phospho-Thr181-Tau levels with disease onset in Alzheimer's disease (Hansson et al., 2006; Fagan and Holtzman, 2010; Hampel et al., 2010). Currently, work is ongoing to evaluate longitudinal CSF samples to identify disease progression biomarkers that correlate with cognition and brain magnetic resonance imaging changes in Alzheimer's disease subjects (Mattsson et al., 2009; Trojanowski et al., 2010).

CSF bioanalysis has also been used to evaluate brain target engagement for agents that affect brain Aβ synthesis and/or clearance. For example, CSF Aβ is used to assess gamma-secretase inhibitors and anti-Aβ therapies in clinical trials in Alzheimer's disease subjects (Siemers et al., 2006, 2010; Bateman et al., 2009). Given the importance of CSF bioanalysis in human clinical studies, preclinical models wherein CSF can be harvested over time will enable better understanding of disease models and to evaluate novel therapeutic agents. Monkeys and dogs have been used in repeated CSF sampling studies for evaluation of beta and gamma-secretase inhibitors in the CNS (Sankaranarayanan et al., 2009; Cook et al., 2010; Gillman et al., 2010; Portelius et al., 2010). These studies have enabled modeling of pharmacokinetic and pharmacodynamic (PK–PD) relationships for different compounds and to understand mechanism of action. While repeated CSF sampling is feasible in large animals, this has been more challenging in rodents.

Abbreviations: Aβ, amyloid beta; CNS, central nervous system; CSF, cerebrospinal fluid; GSI, gamma-secretase inhibitor; PD, pharmacodynamic; PK, pharmacokinetic.

* Corresponding author at: RY80M-124, 126 E. Lincoln Ave., PO Box 2000, Rahway, NJ 07065-0900, USA. Tel.: +1 732 594 6224.
E-mail address: jennifer.shapiro@merck.com (J.S. Shapiro).
[1] Present address: Prospect Farm Drive, Yardley, PA 19067, USA.
[2] Present address: Bristol-Myers Squibb, 5 Research Pkwy, Wallingford, CT 06033, USA.
[3] These authors contributed equally to this work.

0165-0270/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.12.010

Numerous studies have demonstrated CSF Aβ lowering in rats using independent groups of animals to attain temporal relationships of Aβ lowering agents (Best et al., 2005; El Mouedden et al., 2006). In this study, we sought to derive time course relationship of CSF Aβ lowering in rats using repeated CSF sampling in individual animals following compound treatment. Repeated CSF sampling enables temporal characterization of bioanalytes in the same animal, thereby reducing animal usage and minimizing the impact of inter-animal variability on endpoints.

CSF collection from rodents has been performed via the cisterna magna or the lumbar cistern. Cisterna magna CSF sampling has been accomplished by transcutaneous needle access via the back of the neck or by surgical transcranial methods. The transcutaneous approach includes both direct needle access (Takasugi et al., 2005; Nirogi et al., 2010) or open surgical access to the cisterna magna in the back of the neck (Pegg et al., 2010). In contrast, surgical transoccipital bone approach (Sarna et al., 1983; Consiglio and Lucion, 2000) or catheterization via the atlanto-occipital membrane in the back of the neck (Huang et al., 1996) has been used to permanently implant catheters into the cisterna magna to enable repeated CSF sampling. Some reports have also used an indwelling cannula in the lateral ventricle to sample CSF over time via parietal bone access (Cassar et al., 2010). While previous work has shown that chronic, repeated sampling or administration of compounds is feasible in rodents by surgical or transcutaneous approaches, extensive characterization of the intra-animal and inter-animal variability and applicability in pharmacological studies for CNS Aβ lowering agents is lacking.

In this study, we have developed a modification of a transcranial surgical procedure to implant catheters in the cisterna magna (Sarna et al., 1983). Implanted cisterna magna catheters were patent for up to 2 weeks and enabled repeated CSF sampling. CSF Aβ levels showed good intra-animal variability over repeated draws and enabled us to test the time course of gamma-secretase inhibition. We observed a robust and rapid lowering of CSF Aβ40 that recovered over 24–96 h after dosing. Brain Aβ40 and 42 levels measured in terminal samples showed excellent correlation with CSF Aβ40 and 42 levels confirming that CSF Aβ levels closely track brain Aβ levels in this model. Thus, we have validated a model for repeated CSF sampling in rats with broad applicability for diverse CNS pharmacological targets.

2. Materials and methods

2.1. Cisterna magna cannulated rat model

The cisterna magna was cannulated using modification of methods previously described by Sarna et al. (1983). Sprague-Dawley rats (250–350 g) were anesthetized and the hair over the head shaved all the way back to the inter-scapular region. The animal was immobilized in a sterotaxic apparatus under isoflurane anesthesia. An incision was made along the midline to expose the skull from lambda to the occipital crest. The scalp was pushed back and the skull was cleaned and dried. A high speed micro drill with 0.7 mm stainless steel burr (19008-07; Fine Science Tools Inc., Foster City, CA) or a 1/32″ carbide hand drill (2841A92; McMaster-Carr Supply Company) was used to make two holes in the skull. One hole was located 2–3 mm anterior to the occipital crest along the midline. A groove was formed in the skull anterior to this hole to form a guide channel for the dwelling cannula to rest in. A second hole was made a few millimeters anterior and lateral to the first hole to accept an anchor screw (7431021; CMA/Microdialysis AB, Stockholm, Sweden). Catheters were formed from polyethylene-50 tubing (427411; Becton Dickinson, Franklin Lakes, NJ) and threaded with a 30 gauge stainless steel wire to serve as a trocar (13082; Ted Pella, Inc., Redding, CA). A bead was made in the PE tubing after threading with the trocar, by gently heating it. This served as a stop for the catheter during implantation. The catheter tubing was cut ~8–10 mm on one side of the bead while the other end was kept longer until after implantation. The short end of catheter was gently threaded into the first hole, guided posteriorly above and behind the cerebellum so as to reach the cisterna magna. The bead acted as a stop and also helped to secure the catheter in place. The wire served to stiffen the tubing and also helped to puncture the dura at the cisterna magna. After positioning the catheter, the wire was removed and one could observe clear fluid moving up through the tubing. The catheter was flushed with saline and trimmed as necessary. Dental cement (38019; 3M ESPE, St. Paul, MN or 666200; Dentsply International, York, PA) was applied around the catheter and the screw to hold it in place. After the cement dried, the incision was closed and sealed with Nexabond tissue glue and sutured. Excess tubing was cut off leaving ~1.5 inches externally (total internal volume of catheter was ~10 μL). The open end of the catheter was plugged with a removable 23 gauge stainless steel wire (23-PP; Braintree Scientific, Inc., Braintree, MA). The rats were removed from the stereotaxic apparatus and allowed to recover from anesthesia. They were returned to their home cages and individually housed until the end of the study. All animal procedures were fully approved by the Institutional Animal Care and Use Committee at Merck.

2.2. CSF sample collection, animal dosing and euthanasia

Cerebrospinal fluid was collected at different time points starting on the afternoon of the day of surgery. Additional samples were collected for 2 days after surgery prior to dosing of compound. Typically CSF samples were collected at −42, −25, −18, −2, −0.25 h prior to intraperitoneal (IP) dosing of 10 mg/Kg of gamma-secretase inhibitor (GSI) or vehicle. Rats that were not patent following surgery or had blood tinged CSF samples in the first 2 days after surgery were not used further. CSF was collected at 2, 4, 6, 24, 30 h after dosing. The first drop of CSF (~10 μL) was discarded to account for dead volume in tubing. The catheters were flushed with saline after each collection and the cannula capped between sampling time points. Rats were euthanized using a CO_2 chamber at 24 or 96 h after dosing. Terminal CSF samples were collected by accessing the cisterna magna from the back of the neck using a 25 gauge needle coupled to tubing and a 1 mL syringe to apply negative pressure. The collected CSF was spun in pulse mode for three seconds in a microcentrifuge after which the supernatant was removed and stored. In terminal studies, blood was collected by cardiac puncture and transferred to K_2EDTA tubes. Plasma samples were obtained after spinning at 1000× *g* for 15 min. Brains were removed, sectioned mid-sagitally, and each hemisphere was weighed and stored in polypropylene tubes at −80 °C until further analysis.

3. Antibodies and compound

Purified polyclonal antibody against the n-terminus of rodent Aβ (SIG-39153) and 4G8 antibody to amino acids 17–24 of Aβ (SIG-39220) and the Aβ42-neoepitope specific antibody–clone 12F4 (SIG-39142) were from Covance Research Products, Princeton, NJ. Monoclonal Aβ40 specific neoepitope antibody was used similar to that described previously (Ida et al., 1996). The Aβ40 and 42 specific antibodies were conjugated with alkaline phosphatase (AP) using the EZ-link malemide-activated AP kit (31493; Pierce Chemical, Rockford, IL) and used as detection antibodies in ELISA.

The gamma-secretase inhibitor used in this study is a sulfone – (3R,4aS,6S,8aR)-6-(2,5-difluorophenyl)-3-ethyl-6-{[4-(trifluoromethyl)phenyl]sulfonyl}octahydro-1H-2,1-benzothiazine 2,