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Original article

Method for the determination of the levels of β-amyloid peptide in the CSF sampled from freely moving rats

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Abstract

Introduction: In the present study, a model was developed to determine the effect of secretase inhibition on β-amyloid peptide (Aβ) levels in the cerebrospinal fluid (CSF) of freely moving adult rats. **Methods:** Rats were chronically implanted with a cannula into the cisterna magna and CSF samples were collected at different time points from the same animal without anaesthesia. The levels of CSF Aβ were measured by a sandwich ELISA assay. **Results:** Administration of DAPT, a functional γ-secretase inhibitor, resulted in a substantial reduction of Aβ40 and Aβ42, confirming the in vivo functionality of the CSF as a biomarker source for endogenous APP processing modulation by secretase inhibitors. **Discussion:** Thus, the present work provides clear evidence for the usefulness of CSF sampling from the freely moving rat model for testing the effectiveness of small molecule inhibitors of Aβ production.

Keywords: Alzheimer; Amyloid; Cannula; Cisterna magna; CSF sampling; ELISA; Secretase

1. Introduction

Alzheimer's disease (AD) is a dementing neurodegenerative disorder characterized pathologically by major degeneration of the brain cortex with the presence of extracellular senile plaques, which consist primarily of β-amyloid (Aβ) peptides (Blacker & Tanzi, 1998; Hardy & Higgins, 1992).

Aβ is shown to be present in cultured cell media, plasma, cerebrospinal fluid and various tissues (Seubert et al., 1992), indicating that production and release of Aβ peptides is a normal physiological function. However, accumulation and deposition of aggregated Aβ peptides in the form of plaques in the brain is believed to be linked to the pathogenesis of AD (Selkoe, 2001).

Aβ peptides are cleaved from the large transmembrane amyloid precursor protein (APP) by two proteases termed β-secretase and γ-secretase. APP is first cleaved by the β-secretase, BACE1, at the N-terminus of the Aβ domain (Selkoe, 2001). This cleavage generates the soluble sAPPβ and a C-terminal fragment, which undergoes a second cleavage by a protease called γ-secretase. BACE1 is a single membrane-spanning aspartyl protease expressed at high levels in neurons (Vassar & Citron, 2000). γ-secretase is also an aspartyl protease (Wolfe, De Los Angeles, Miller, Xia, & Selkoe, 1999; Wolfe & Selkoe, 2002) but with a novel intramembranous catalytic site that is required for the cleavage of a wide range of type I membrane protein substrates that includes APP and the Notch receptors (Kopan & Ilagan, 2004). γ-secretase activity is associated with a high molecular weight protein complex composed of at least four transmembrane proteins, i.e. presenilin (PS), which is predicted to harbor the catalytic site as an aspartic protease, as well as nicastrin (NCT), anterior pharynx defective-1 (APH-1), and presenilin enhancer-2 (PEN-2) as essential transmembrane cofactor proteins (De Strooper, 2003; Wolfe, 2002). The fact that mice deficient in either protease do not generate the Aβ peptide clearly implicates BACE1 and the γ-secretase complex as the amyloidogenic

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proteases in vivo and makes them suitable drug targets for preventing or slowing down AD (Dewachter et al., 2002; Luo et al., 2001). Moreover, many studies have shown that treatment with active or passive immunization regimen that consisted of anti-Aβ antibodies resulted in a dramatic reduction in total Aβ and reversal of memory deficits in the APP transgenic mice (DeMattos et al., 2001; Dodart et al., 2002; Janus et al., 2000; Morgan et al., 2000).

Cerebrospinal fluid is in direct contact with the brain extracellular space and reflects the extracellular milieu of the central nervous system (CNS) (Burns et al., 1976). Therefore, multiple sampling of CSF can be used to evaluate the relationship between pharmacological treatment and changes to APP processing. The most often CSF sampling methods used are the cisternal puncture (Chou & Levy, 1981) and the cisternal cannulation (Bouman & Van Wimersma Greidanus, 1979; Kiser, 1982; Kornhuber, Kornhuber, & Cimniak, 1986; Sarna, Huston, Tricklebank, & Curzon, 1983; Westergren & Johansson, 1991). In the first method, an incision is made in the skin overlying the atlanto-occipital membrane and the CSF is sampled by inserting a needle through the atlanto-occipital membrane into the cisterna magna. In the second method, a cannula is implanted through the roof of the skull along the cerebellum into the cisterna magna. Both approaches sample CSF from the cisterna magna, the largest CSF compartment lying between the cerebellum and the first cervical vertebra. The former method is terminal for the animal, while the second showed a lot of advantages: accurate sampling without loss and contamination of blood in CSF, multiple sampling across a time period in awake animals, (anaesthesia is not required) and the sampling method is not terminal for the animal.

The aim of the present study was to develop a suitable rat model to investigate, at different time points, in CSF, endogenous APP processing modulation by small molecule inhibitors of secretases. For that purpose, the cannulation through the interparietal bone using a stereotaxic frame was performed and CSF was collected to monitor Aβ levels.

2. Methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River Germany), weighing 250–300 g, were used. Tap water and food were available ad libitum.

2.2. Anaesthesia

Tracheal intubation was performed under 4% isoflurane in a mixture of 30% O_2 and 70% N_2O (1000 ml/min). Anaesthesia was then maintained at 1.5% isoflurane in a mixture of 30% O_2 and 70% N_2O (1000 ml/min) for the duration of the surgical procedure.

2.3. Surgical implantation of cannula

The rat head was placed with the skull in a horizontal position (3.3 mm under the inter-aural line) in a stereotaxic apparatus (David Kopf instruments). A 3 cm incision was made in the skin at the back of the head and the overlaying connective tissue was removed to expose the skull. A small hole was drilled in the skull on the sagittal midline immediately rostral to the interparietal–occipital bone structure using a dental Trephine drill with a diameter of 2.3 mm. The hole was drilled in such a way that the occipital bone could be used as a guide while inserting the cannula. An additional three holes were made in the parietal bone, using a drill of 1.2 mm diameter, for the placement of three stainless screws (A.D. Leydens Belgium, 0.1 mm diameter and 3.0 mm length) for additional anchoring of the cannula. The cannula was slowly placed into the cisterna magna to minimize the damage in the cerebellum (Fig. 1B). The correct placement

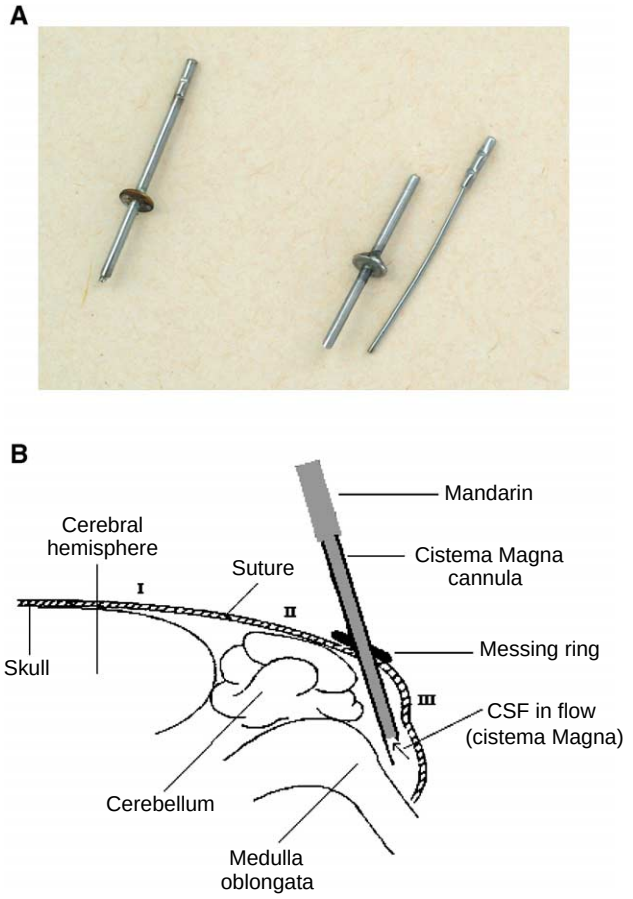

Fig. 1. A photograph of the cannula and the schematic drawing of the technique used for sampling CSF from the cisterna magna in adult rats. (A) The cannula of 17 mm length and the mandarin of 0.6 mm diameter were made as described in Methods. (B) The cannula was inserted through a hole drilled in the skull, forced epidurally into the cisterna magna and fixed to the bone skull with dental cement. The other end of the tube was externalised. I: parietal bone, II: interparietal bone, III: occipital bone.