Cerebrospinal fluid and plasma distribution of myo-inositol and other polyols in Alzheimer disease

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Previous studies suggest the presence of increased concentrations of cerebral myo-inositol in Alzheimer disease (AD). To characterize this abnormality further, we quantified myo-inositol and several other polyols in cerebrospinal fluid (CSF) and plasma from 10 AD subjects and 10 healthy age-matched controls by using a gas chromatographic–mass spectrometric technique. The mean CSF concentration and CSF/plasma concentration ratio of myo-inositol in AD were not significantly different from those determined in control subjects. Also, concentration profiles of other polyols were not significantly altered in AD. CSF and plasma myo-inositol concentrations were correlated in control subjects but not in AD subjects. However, a significant correlation between CSF and plasma 1,5-anhydroinositol (a polyol internal control) concentrations observed in control subjects was retained in AD subjects.

INDEXING TERMS: carbohydrate metabolism • gas chromatography–mass spectrometry

Cellular homeostasis of myo-inositol is critical for optimal functioning of neurons. Its disruption may lead to neurological complications [1,2]. In its major functional role, myo-inositol is incorporated into membrane, and the product, phosphatidylinositol, after phosphorylation, is a source of the second messenger inositol trisphosphate, released in a signal-transduction pathway [3]. To maintain this dynamic role and other roles such as osmotic volume regulation in brain [4], the concentration of myo-inositol must be tightly regulated by active transport and de novo synthesis. Altered concentrations of myo-inositol and other polyols are known to perturb physiological properties of nerves. A decrease in sciatic motor-nerve conduction velocity was observed when the myo-inositol concentration was experimentally increased in animals [2]. In diabetic neuropathy, an increase in sorbitol and a decrease in myo-inositol concentrations correlate with decreased Na⁺/K⁺-ATPase activity and changes in electrical properties of neurons [1].

Using a gas chromatographic–mass spectrometric (GC-MS) technique to measure polyol species, we recently found 50% increases in the cerebrospinal fluid (CSF) concentration and the CSF/plasma concentration ratio (RCSF) of myo-inositol in adult Down syndrome subjects [5]. There was no difference in the mean concentration between younger (<35 years) and older Down syndrome subjects. These results are consistent with an increase in the intracerebral myo-inositol/creatinine ratio determined by others with an in vivo magnetic resonance spectroscopy method [6]. Accumulation of myo-inositol in Down syndrome may be the result of a gene dosage effect, as a Na⁺/myo-inositol cotransporter gene (SLC5A43) has been localized to the long arm of chromosome 21 [7].

In Alzheimer disease (AD), a recent in vivo magnetic resonance spectroscopy study indicated above-normal concentrations of brain cerebral myo-inositol [8]. However, a previous study on postmortem AD brain showed no such increase [9]. At a functional level, inositol triphosphate-mediated Ca²⁺ release, subsequent to phospholipase C activation by bombesin, is reported to be greatly enhanced in AD fibroblasts compared with fibroblasts from control groups [10]. Thus, to gain an insight into the possible alterations in myo-inositol metabolism and carbohydrate metabolism in general, we quantified myo-inositol and several other polyol species in CSF and plasma from AD subjects.

Materials and Methods

Materials. Polyol species were purchased from Sigma Chemical Co., St. Louis, MO. Deuterium-labeled myo-inositol (³H₂myo-inositol) was from Merck Sharp & Dohme/Isotopes, Pointe Claire-Dorval, PQ, Canada. Pyridine and acetic anhydride were obtained from Alltech Associates, Deerfield, IL, and solvents were from Burdick & Jackson, Muskegon, MI. The GC-MS was ITSY40™ (Finnigan MAT, San Jose, CA). The capillary column (Rtx 50) was from Restek, Bellefonte, PA.

1 Nonstandard abbreviations: CSF, cerebrospinal fluid; AD, Alzheimer disease; GC-MS, gas chromatography–mass spectrometry; and RCSF, CSF/plasma ratio.
**Selection of subjects.** For this study, we chose 10 subjects, 7 men and 3 women, ages 47–76 (62.1 ± 9.9, mean ± SD) years, who met DSM III-R [11] criteria for dementia and research diagnostic criteria for probable AD according to NINCDS-ADRDA [12] guidelines. The subjects had been demented for 5 to 9 years and their MiniMental State Examination scores [13] ranged from 1 to 25. Seven of the selected subjects have since come to autopsy and each showed histopathology consistent with diagnostic criteria for AD [14]. The control group consisted of 10 subjects, 6 men and 4 women, ages 50–92 (64.4 ± 12.4) years. The AD and control subjects in this study were enrolled in longitudinal studies on dementia and aging, respectively, in the Laboratory of Neurosciences, National Institute on Aging. The study of AD and control subjects was approved by the Institutional Review Board of the National Institute on Aging. Consent was obtained from each subject (and their conservator, where appropriate) after a full explanation of the purpose, procedures, and risks of the study.

All subjects underwent a review of medical history, physical and neurological examinations, and a psychiatric evaluation. Medical screening included blood tests, electroencephalograms, magnetic resonance imaging of brain, chest x-ray, electrocardiograms, audiological assessments, a MiniMental State Examination [13], the Hachinski Ischemia Scale [15], Hamilton Depression Scale [16], and ratings of extrapyramidal signs and ability to perform daily living activities. Subjects were excluded if they had a history of significant medical, neurological, and psychiatric illness, including neoplastic disease, epilepsy, stroke, traumatic head injury, and alcohol/drug abuse. There was no evidence of significant cerebrovascular disease, lacunar infarcts, or mass effects on magnetic resonance scans of the head.

**Sample collection.** The subjects had taken no medication for at least 2 weeks before lumbar puncture. For 72 h they were placed on a diet that was low in monoaminergic precursors [17] and were on overnight bed rest and fasting. The following morning, lumbar punctures were performed with subjects in the lateral decubitus position. After a sterile preparation, the L3–4 interspace was infiltrated with lidocaine (10 g/L). A 20-gauge spinal needle then was inserted into the spinal subarachnoid space. Specimens were analyzed only if the CSF was clear, with normal cell counts, protein, and glucose. The first 12 mL of CSF collected were pooled, immediately placed on wet ice, and frozen in 1-mL aliquots at −80 °C until assayed. Just before the lumbar puncture, venous blood was withdrawn through an indwelling intravenous catheter placed 10 h before (to minimize acute changes of blood and CSF neurotransmitters). Stable blood pressures and heart rates were recorded over three or more successive 10-min periods before venous sampling. These measurements were made to assess the level of stress, which could in turn influence the concentrations of some neurotransmitters. The blood was placed in heparinized polypropylene tubes and immediately centrifuged at 5000g at 4 °C for 20 min. One-milliliter aliquots of the resulting plasma were removed and stored at −80 °C until assayed.

**GC-MS assay of polyol.** Polyl species in CSF and plasma were quantified, as outlined here, by a GC-MS method developed in our laboratory [18]. CSF (25 µL) or plasma (100 µL) was mixed with the internal standard ([2H6]myo-inositol), treated with methanol, and centrifuged; the supernate was removed and evaporated. After the polyols in the samples were acetylated with acetic anhydride/pyridine/4-dimethylaminopyridine, the reaction products were dissolved in hexane/ethyl acetate and then washed with sodium bicarbonate solution. The organic layer was evaporated to dryness, and the residue was reconstituted in ethyl acetate. An aliquot of this solution was injected into the GC-MS. The components were resolved on a capillary column (bonded 50% phenyl–50% methyl polysiloxane), and individual polyol species were detected and quantified by a chemical ionization technique in an ion trap mass spectrometer. Each polyol yielded a highly abundant fragment ion corresponding to the loss of one CH3COOH residue from the protonated molecule. The ions monitored were m/z 273 for 1,5-anhydro-d-sorbitol; m/z 303 for ribitol, arabitol, and xylitol (stereoisomers); m/z 373 for myo-inositol; and m/z 375 for mannitol, sorbitol, and galactitol (stereoisomers). The hexa-deuterated myo-inositol (internal standard) yielded an m/z 379 ion, which was acquired simultaneously. The concentration of each polyol species in CSF or plasma was read from the calibration curve generated separately. The relative standard deviation (CV) for quantification was not more than 8% for CSF polyols and 15% for plasma polyols.

**Statistical analysis.** The mean concentration of each polyol in CSF or plasma of the AD group was compared with the respective mean of the control group by a two-tailed t-test. R_{CSF} was calculated for each subject, and the mean values were similarly compared. The statistical difference between mean R_{CSF} and a hypothesized mean 1.0 was determined by a one-sample t-test. A correlation analysis on variables was performed by linear regression. The criterion for statistical significance was P <0.05.

**Results**

Several polyol species in CSF or plasma were simultaneously quantified by GC-MS with deuterium-labeled myo-inositol as the internal standard. Ion chromatograms for CSF and plasma polyols from an AD subject are shown in Fig. 1. The amount of xylitol is negligibly low in CSF from both AD and control subjects, so this species was not quantified. There was no apparent difference in the chromatographic profile of CSF or plasma polyols between AD and control subjects. Concentrations of CSF polyols in controls and AD subjects are shown in Table 1. The mean CSF concentration of myo-inositol, 28.6 ± 4.9 mg/L (mean ± SD), in AD was not significantly (P = 0.087) different from the 24.9 ± 4.4 mg/L found in control subjects. Similarly, mean concentrations of ribitol, arabitol, 1,5-anhydro-d-sorbitol, mannitol, sorbitol, and galactitol in AD were not significantly different from those of control subjects. The plasma concentrations of polyol species in AD and control subjects are also shown in Table 1. None of the concentrations of these sugar metabolites was significantly altered in AD.
The myo-inositol concentrations significantly arabitol, the polyols were divided into several categories: ribitol, arabitol, sorbitol, and galactitol. Arabitol, galactitol, and ribitol, and myo-inositol, both in AD and control subjects. The CSF:plasma concentration ratios were calculated for each polyol in both control and AD subjects. However, plasma and CSF concentrations of 1,5-anhydroxylitol showed correlation in control subjects (r = 0.69, P < 0.05), remarkably, no correlation was found in AD subjects. In AD, mean R_{CSF} values of the polyols in CSF was significantly different from those for control subjects.

No correlation was found between plasma and CSF concentrations for the polyols mannitol, arabitol, ribitol, and arabinosylcellulose (CSF). This suggests that the metabolism of these polyols may be affected if, for instance, the uptake and utilization of sugars are skewed. In metabolic disorders such as diabetes and galactosemia, accumulation of polyols occurs in various tissues, including brain [1, 19]. Thus, measurement of polyols in CSF and plasma compartments could aid in our understanding of the metabolism (synthesis, transport, etc.) of polyol species themselves as well as of sugar precursors in the central nervous system.

The concentrations of ribitol, Arabitol, myo-inositol, manni-

| Table 1. Mean ± SD polyol concentrations in CSF and plasma from control and Alzheimer subjects. |
|---|---|---|---|
| Polyl | Control subjects | Alzheimer subjects | Control subjects | Alzheimer subjects |
| Mannitol | 0.999 ± 0.309 | 1.01 ± 0.15 | 0.438 ± 0.302 | 0.445 ± 0.233 |
| Sorbitol | 3.54 ± 1.03 | 4.17 ± 0.61 | 0.253 ± 0.128 | 0.215 ± 0.041 |
| Galactitol | 0.331 ± 0.082 | 0.290 ± 0.038 | 0.064 ± 0.036 | 0.057 ± 0.024 |
| Ribitol | 0.555 ± 0.102 | 0.578 ± 0.103 | 0.083 ± 0.012 | 0.083 ± 0.012 |
| Arabitol | 4.37 ± 1.32 | 3.42 ± 0.73 | 0.481 ± 0.115 | 0.490 ± 0.116 |
| Xyitol | 18.2 ± 5.0 | 16.1 ± 5.3 | 0.160 ± 0.044 | 0.136 ± 0.034 |
| 1,5-Anhydroxylitol | 24.9 ± 4.4 | 28.6 ± 4.9 | 20.7 ± 4.9 | 19.9 ± 5.0 |
| myo-Inositol | 24.9 ± 4.4 | 28.6 ± 4.9 | 4.30 ± 1.30 | 4.48 ± 1.15 |

n = 10 each group.

| Table 2. Mean ± SD CSF:plasma concentration ratios, R_{CSF}, for polyols. |
|---|---|
| Polyl | Control subjects | Alzheimer subjects |
| Mannitol | 2.68 ± 0.91 | 2.73 ± 1.18 |
| Sorbitol | 16.9 ± 9.3 | 20.4 ± 6.8 |
| Galactitol | 8.31 ± 1.77 | 6.37 ± 3.66 |
| Ribitol | 8.61 ± 1.67 | 7.01 ± 1.48 |
| Arabitol | 9.27 ± 2.69 | 7.25 ± 1.98 |
| Xyitol | 1.19 ± 1.00 | 1.00 ± 1.00 |
| 1,5-Anhydroxylitol | 0.884 ± 0.240 | 0.799 ± 0.127 |
| myo-Inositol | 6.06 ± 1.39 | 6.71 ± 1.41 |

n = 10 for each group.

* Significant positive correlation between CSF and plasma concentrations at
a P < 0.05 or c P = 0.001.

**Discussion**

Polyols ribitol, arabitol, xylitol, mannitol, sorbitol, and galactitol are generated by the reduction of their respective sugar precursors. myo-Inositol is synthesized de novo from glucose in an oxidoreductive pathway; the immediate sugar precursor for 1,5-anhydroxylitol is not known. The concentrations of these metabolites may be affected if, for instance, the uptake and utilization of sugars are skewed. In metabolic disorders such as diabetes and galactosemia, accumulation of polyols occurs in various tissues, including brain [1, 19]. Thus, measurement of polyols in CSF and plasma compartments could aid in our understanding of the metabolism (synthesis, transport, etc.) of polyol species themselves as well as of sugar precursors in the central nervous system.

The concentrations of ribitol, arabitol, myo-inositol, manni-

Fig. 1. Ion chromatograms resulting from the GC-MS analysis of (a) CSF polyols and (b) plasma polyols in an AD subject.

Peaks: (1) ribitol, (2) arabitol, (3) xylitol, (4) myo-inositol, (5) 1,5-anhydroxylitol, (6) mannitol, (7) sorbitol, (8) galactitol, and (9) [H\text{H}_6]myo-inositol (internal standard).
tol, sorbitol, and galactitol in CSF are several-fold higher than in plasma, suggesting preponderant brain origin of these CSF metabolites. In general, a $R_{CSF}$ value $>2$ is indicative of brain as the source of the molecule unless contribution from blood to CSF by an active transport is significant [18, 20]. Brain maintains millimolar concentrations of myo-inositol by de novo synthesis [21] as well as by active transport from the plasma source [22]. Both brain and plasma contribute to CSF myo-inositol; however, the plasma contribution is relatively low [23, 24]. In the absence of transporters, the high $R_{CSF}$ found for the majority of the polyols is indicative of a high degree of metabolism of their sugar precursors in the brain. 1,5-Anhydro-sorbitol with its $R_{CSF} < 1$ probably has much of its origin in plasma, as suggested by the correlation between CSF and plasma concentrations. The data on xylitol suggest that it is not partitioned into the CSF compartment to any significant amount from either blood or brain.

In the present study involving 10 subjects in each group, we observed no significant increase in the concentration of CSF myo-inositol in AD. Previous studies with in vivo magnetic resonance spectroscopy indicated a significant increase (50%) in the amount of myo-inositol in the occipital region of the AD brain [8, 25]. The occipital brain region is relatively spared in AD [26]. Regional variation in the concentrations of myo-inositol within brain is well documented [27]. Thus, if the increase in myo-inositol is regional because of upregulation of the local synthesis or transport, that change may not be detected in the CSF pool. On the other hand, the 50% increase in myo-inositol as determined by magnetic resonance spectroscopy requires confirmation by postmortem brain analysis with other techniques. It was not confirmed in one such study [9]. Also, in the magnetic resonance spectroscopy method, isolation of a resonance signal at the chemical shift of a myo-inositol proton without interference by other molecules in the unresolved complex matrix is difficult to achieve. Moreover, the signal for free myo-inositol may not be resolved from those of its metabolites.

Other polyols also were not affected in CSF in AD, suggesting that there is no significant alteration in the metabolism of their sugar precursors and, possibly, no significant alteration of carbohydrate metabolism in general. Because plasma concentrations of polyols were also not affected, no significant alteration in $R_{CSF}$ was detected in AD. These results are in contrast to the significant alterations in the $R_{CSF}$ of myo-inositol, ribitol, and arabitol observed in Down syndrome [5], although the deviations in $R_{CSF}$ found were unrelated to dementia in Down syndrome.

The concentrations of CSF and plasma myo-inositol were significantly correlated in control subjects. The loss of this correlation in AD is intriguing. Moreover, the correlation for 1,5-anhydro-sorbitol (a polyol internal control) was retained in AD, similar to the situation in Down syndrome. A lack of correlation between CSF and plasma concentrations of myo-inositol was also observed in Down syndrome [5]. Although CSF myo-inositol is predominantly of brain origin, its concentration is influenced by active transport from the plasma source [28]. Thus, the correlation between CSF and plasma myo-inositol concentrations, as observed in control subjects, may be related to the transporter. The correlation may be affected if the relative contribution of myo-inositol to CSF pool by brain is increased over plasma. In Down syndrome, the lack of correlation between CSF and plasma concentrations may be the result of 1.5 times more expression of the Na"/myo-inositol cotransporter gene [7]; the same transporter may be affected in AD brain. Also, altered myo-inositol synthase as well as altered phosphatidylinositol turnover in limited regions of the AD brain may account for this anomaly. Unlike myo-inositol, the polyols ribitol, arabitol, mannotol, sorbitol, and galactitol with $R_{CSF} > 2$ show no correlation between their CSF and plasma concentrations, both in controls and AD subjects. Thus, it is unlikely that transporters are involved in maintaining gradients for these polyols.

The present study does not support magnetic resonance spectroscopy evidence [8] for excess accumulation of myo-inositol in AD brain. Data on myo-inositol and phosphatidylinositol concentrations in various regions of postmortem AD brain with appropriate controls may provide the explanation for the loss of correlation between the CSF and plasma concentrations of myo-inositol. Additionally, analysis of brain tissues by a more specific analytical technique will provide the needed verification of the in vivo magnetic resonance spectroscopy data.

References


