Ts65Dn Mouse, a Down Syndrome Model, Exhibits Elevated myo-Inositol in Selected Brain Regions and Peripheral Tissues

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myo-Inositol is elevated in the Down syndrome (DS; trisomy 21) brain and may play a role in mental retardation. In the present study, we examined brain regions and peripheral tissues of Ts65Dn mouse, a recently characterized genetic model of DS, for abnormal myo-inositol accumulation. A GC/MS technique was used to quantitate myo-inositol and other polyol species (ribitol, arabitol, xylitol, and 1,5-anhydrodorsorbitol) in tissues from the Ts65Dn mice and control diploid mice. myo-Inositol was found to be elevated in frontal cortex, hippocampus, and brain stem but not in cerebellum of the Ts65Dn mouse. Among peripheral organs examined, liver and skeletal muscle were found to excessively accumulate myo-inositol. In all tissues, concentrations of polyol internal controls were normal. The Ts65Dn mouse is useful to study the possible effect of elevated myo-inositol on cellular processes.

KEY WORDS: myo-Inositol; Ts65Dn mouse; Down syndrome; brain; skeletal muscle; liver.

INTRODUCTION

Down syndrome (DS; trisomy 21) has multiple effects, including mental retardation, early-onset Alzheimer pathology, hypotonia, cardiovascular malformations, ocular abnormalities, immunological disorders, thyroid dysfunction, and male sterility. Animal models of DS may help to identify the abnormal molecular processes involved. A segment of mouse chromosome 16 displays genetic homology with human chromosome 21, and thus a full trisomy 16 (Ts16) mouse (1) and, more recently, a segmental trisomy 16 (Ts65Dn) mouse (2) have been characterized as animal models of DS. The Ts16 mouse dies in utero whereas the Ts65Dn mouse survives to adulthood, permitting examination of various tissues for biochemical and physiological defects during development and in a mature state.

In DS, gene products of the extra chromosome 21 may cause abnormal levels of certain metabolites due to altered synthesis, transport, catabolism or regulatory pathways. Some of the metabolites may have a role in the DS pathophysiology. Recently, we screened polyols by mass spectrometry and identified elevated (50%) myo-inositol in DS brains compared with controls (3,4). It appears that the extent of elevation of brain myo-inositol in young DS adults is correlated with impaired cognitive performance (5). The human Na+/myo-inositol cotransporter gene has been localized to the q arm of the chromosome 21 (6) and thus an increased transport may be the mechanism for the brain elevation of myo-inositol.
Cells utilize myo-inositol for osmoregulation and phosphatidylinositol signaling. The possible alterations in these cellular processes remain to be investigated in DS. In addition, myo-inositol levels in brain on a regional basis and in peripheral organs have not been examined in this trisomic disorder. An animal model of DS such as Ts65Dn mouse is helpful in elucidating the molecular and physiological bases for the excessive myo-inositol accumulation. Brain regions and peripheral organs can be conveniently obtained with a minimum postmortem delay to assess the degree of accumulation. In addition, various tissues of the animal model can be used to measure myo-inositol transport and transporter mRNA expression. In the present study, brain regions and peripheral organs of Ts65Dn mice and control diploid mice were analyzed by mass spectrometry and tissue selectivity in the abnormal myo-inositol accumulation was demonstrated.

EXPERIMENTAL PROCEDURE

Ts65Dn Mouse. Diploid control and Ts65Dn mice were obtained by breeding Ts65Dn females with B6C3HF1 males (Jackson Laboratories, Bar Harbor, ME) in our animal care facility, or were obtained directly from Jackson Laboratories. Animals were maintained under a 12-h light/dark cycle and fed standard laboratory chow (NIH-07 guidelines, 41-0710-75-53). Karyotyping was performed with chromosomal spreads from either spleen or peripheral blood samples (7.8). Metaphase chromosome spreads were prepared as previously described (9), and karyotypes were evaluated. Control and Ts65Dn mice were anaesthetized and decapitated (8), and the brain was quickly excised. Selected brain regions and peripheral organs were dissected out and stored at −80°C until analysis. Ts65Dn mice (9 male) were 65 ± 3 (mean ± SEM) days old and control diploid animals (9 male) were 70 ± 3 days old (P > 0.05).

Gas Chromatographic/Mass Spectrometric (GC/MS) Assay of Polyols. Frozen tissue (∼30 mg) from a mouse (Ts65Dn or diploid control) was quickly weighed in a polypropylene tube; [2H6]myo-inositol (internal standard) solution (50 µl of 100 ng/µl) and methanol (0.5 ml) were added and the tissue was homogenized (probe sonicator). The resulting suspension was centrifuged and the pellet was assayed for protein (BCA reagent, Pierce, Rockford, IL), whereas the supernate was assayed for polyols by a method reported from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). Pyridine and acetic anhydride were obtained from Alltech Associates, Inc. (Deerfield, IL), and solvents were from Burdick & Jackson (Muskegon, MI). The gas chromatograph/mass spectrometer was a Finnigan MAT ITS40M (San Jose, CA). The capillary column was from Restek Corporation (Bellevonte, PA).

Statistical Analysis. The mean concentration of each polyol in Ts65Dn mouse tissue was compared with the mean for control mouse tissue by a two-tailed t test. The criterion for statistical significance was P < 0.05.

RESULTS

The GC/MS technique (10) allowed simultaneous measurement of myo-inositol and of other polyols (internal control substances)—ribitol, arabinitol, xylitol, and 1,5-anhydroxylitol—in brain and peripheral tissues. Concentrations of reference polyols are shown only for the frontal cortex (Table I). In this brain region of the Ts65Dn mouse, myo-inositol was found to be elevated (30%), without there being a significant change in the other polyols. Each mg (weight) of frontal cortex yielded 72.0 ± 3.8 (mean ± SEM) µg protein for control mice and 65.0 ± 3.1 µg protein for Ts65Dn mice (P > 0.05). Fig. 1 shows regional concentrations of myo-inositol in brain of control and Ts65Dn mice. myo-Inositol was significantly elevated in hippocampus (28%), brain stem (21%), and frontal cortex. In contrast, myo-inositol was not altered in the Ts65Dn cerebellum. Concentrations of the reference polyols were not altered in frontal cortex, hippocampus, brain stem, or cerebellum of the Ts65Dn mouse. Significant regional variation in the concentration of

<table>
<thead>
<tr>
<th>Polyols</th>
<th>Control (n = 9)</th>
<th>Mean ± SEMa</th>
<th>Ts65Dn (n = 9)</th>
<th>Mean ± SEMa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribitol</td>
<td>14.18 ± 0.49</td>
<td>14.28 ± 0.43</td>
<td></td>
<td></td>
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<tr>
<td>Arabitol</td>
<td>50.51 ± 3.14</td>
<td>47.72 ± 2.07</td>
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<tr>
<td>Xylitol</td>
<td>14.88 ± 0.97</td>
<td>16.39 ± 0.91</td>
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<td></td>
</tr>
<tr>
<td>1,5-Anhydroxylitol</td>
<td>19.07 ± 2.04</td>
<td>17.32 ± 1.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>9.50 ± 0.31 × 103</td>
<td>12.37 ± 0.38 × 103</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Concentrations in ng/mg protein
* P < 0.0001

and m/z 373 for myo-inositol, were monitored. The constituent ion m/z 379 of the internal standard was acquired simultaneously. The concentration of each polyol species in tissue was read from a corresponding standard curve generated using a mixture of polyol standards.

Materials. Polyol standards were obtained from Sigma Chemical Co. (St. Louis, MO). The deuterium-labeled internal standard was from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). Pyridine and acetic anhydride were obtained from Alltech Associates, Inc. (Deerfield, IL), and solvents were from Burdick & Jackson (Muskegon, MI). The gas chromatograph/mass spectrometer was a Finnigan MAT ITS40M (San Jose, CA). The capillary column was from Restek Corporation (Bellevonte, PA).

Table I. Concentrations of myo-Inositol and Internal Controls in the Frontal Cortex of Control and Ts65Dn Mice

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