RESEARCH COMMUNICATION

Brain accumulation of *myo*-inositol in the trisomy 16 mouse, an animal model of Down's Syndrome

H. Umesha SHETTY*, Harold W. HOLLOWAY, Larisa D. ACEVEDO and Zygmunt GALDZICKI

Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, U.S.A.

myo-Inositol and several other polyols were measured in the tissues of the trisomy 16 mouse (animal model of Down's Syndrome; human trisomy 21) and diploid controls. *myo*-Inositol was found to be selectively elevated in the brain of the trisomy 16

mouse. However, peripheral tissues showed no elevation. These results are consistent with the cerebrospinal fluid and plasma data reported previously on *myo*-inositol in Down's Syndrome subjects.

INTRODUCTION

The Down's Syndrome (DS) (human trisomy 21) phenotype is composed of diverse structural and functional abnormalities. This phenotype results from expression of three copies of chromosome 21 genes. Thus, mental retardation, cardiovascular malformations, ocular abnormalities, immunological disorders, leukaemia, early-onset Alzheimer's pathology and other abnormalities present in DS are the manifestations of gene dosage effects. Many genes on chromosome 21 and their protein products have been identified. However, the molecular events related to excess gene products, responsible for ensuing developmental abnormalities and for a multitude of pathophysiological abnormalities characteristic of DS, remain to be elucidated. To understand these abnormalities in molecular terms, metabolites that are vital for normal cellular function and integrity, along with protein products, need to be examined. Cellular homeostasis for certain small molecules may be disrupted, secondary to an increased gene dosage in DS.

In DS, if a gene for a transporter or for an enzyme in a metabolic or regulatory pathway is localized to chromosome 21, its increased expression could alter the homeostasis of metabolites involved. When polyol species (reduction products of sugars) were examined in cerebrospinal fluid (CSF), a significant increase (49%) in the level of *myo*-inositol alone was observed in both young-adult and older DS subjects [1]. The increase was unrelated to age. Also, plasma *myo*-inositol was unchanged in these subjects and the CSF-to-plasma concentration ratio was significantly increased, suggesting the brain as the origin of elevated CSF *myo*-inositol. Recent mapping of an Na⁺/*myo*-inositol co-transporter gene (SLC5A3) [2] onto the long arm of chromosome 21 suggests furthermore that the altered *myo*-inositol homeostasis involving the CSF results from increased transport.

A portion of mouse chromosome 16 is genetically homologous to the long arm of human chromosome 21, resulting in several comparable anatomical, biochemical and pathophysiological abnormalities in the trisomic state [3,4]. Thus, the trisomy 16 (Ts16) mouse is regarded as an animal model of DS. These mice do not survive the full gestation period, but their fetuses have

been used to study certain aspects of the DS phenotype. In the present study, *myo*-inositol and other polyols in brain and peripheral tissues of the Ts16 mouse fetus were analysed and compared with levels in control tissue, to determine whether a *myo*-inositol abnormality is present in the Ts16 mouse consistent with that reported in DS [1].

MATERIALS AND METHODS

Animal and tissue preparations

Ts16 mouse fetuses were obtained by breeding males that were doubly heterozygous for Robertsonian translocations of chromosome 16 [Rb(16.17)32Lub/Rb(11.16)2H] with normal C57BL/6 females [5]. After 15–16 days of gestation, females were killed by CO₂ asphyxiation and the fetuses were removed. Normal diploid sibling fetuses were used as controls. Fetal brains were dissected out and the meninges removed. The brains and torsos of the animals were frozen on dry ice. The postmortem delay was less than 2 min. Ts16 mouse fetuses were identified by their characteristic anasarca and retarded development. Chromosome spreads were prepared to confirm correct selection.

Gas chromatographic/mass spectrometric assay of polyols

Frozen tissue (20–30 mg of brain or 100–250 mg of torso) from Ts16 or diploid mouse was quickly weighed in a polypropylene tube. Following addition of internal standard, [³H]₆*myo*-inositol (100 µl of 100 ng/µl) and methanol (0.5 ml), the tissue was homogenized (probe sonicator). The resulting suspension was centrifuged and the polyols in the supernatant (50 µl) were quantified by a method described previously for polyols in CSF and plasma matrices [6]. Briefly, the supernatant was evaporated to complete dryness. The residue was heated with acetic anhydride/pyridine/4-dimethylaminopyridine, dissolved in hexane/ethyl acetate and 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 a gas chromatograph/mass spec-

trometer. Polyols were resolved on a capillary column (bonded 50 % phenyl–50 % methyl polysiloxane) and individual 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 CH_3COOH residue from the protonated molecular ion. The ions: m/z 273 for 1,5-anhydrosorbitol; 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), were monitored. The constituent ion, m/z 379, of the internal standard was acquired simultaneously. The concentration of each polyol species in the 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, U.S.A.). ^3H -Labelled internal standard was from Merck Sharp and Dohme/Isotopes (Point Claire-Dorval, Quebec, Canada). Pyridine and acetic anhydride were obtained from Alltech Associates, Inc. (Deerfield, IL, U.S.A.) and solvents were from Burdick and Jackson (Muskegon, MI, U.S.A.). The gas chromatograph/mass spectrometer was a Finnigan MAT ITS40 (San Jose, CA, U.S.A.). The capillary column was from Restek Corporation (Bellefonte, PA, U.S.A.).

Statistical analysis

The mean concentration of each polyol in brain or peripheral tissues of the Ts16 mouse was compared with the respective mean in the diploid mouse by a two-tailed *t*-test. The criterion for statistical significance was $P < 0.05$.

RESULTS AND DISCUSSION

Quantitative data on polyol species in brain tissues (wet) from Ts16 and control, diploid mice are shown in Table 1. The mean concentrations of mannitol, sorbitol, ribitol, arabitol, xylitol and 1,5-anhydrosorbitol in the Ts16 mouse were not significantly different from those in the diploid mouse. These results possibly reflect unchanged uptake, utilization and metabolism of sugar precursors of polyols in the fetal Ts16 mouse brain. Only the concentration of *myo*-inositol, 699 ± 149 ng/mg, in Ts16 mouse brain was significantly ($P < 0.005$) higher (by 33 %) than that found in diploid mouse, 526 ± 127 ng/mg. In DS, the CSF level of *myo*-inositol was increased by 49 % [1]. Also, in DS no other CSF polyol was elevated. Thus, the results are analogous.

Table 1 Polyol concentrations in brain of fetal mouse

Polyols	Diploid ($n = 14$) Mean $\pm$ S.D. (ng/mg)	Trisomy 16 ($n = 14$) Mean $\pm$ S.D. (ng/mg)
Mannitol	1.05 ± 0.49	0.929 ± 0.361
Sorbitol	5.37 ± 2.23	4.87 ± 1.11
Galactitol	NQ*	NQ*
Ribitol	0.994 ± 0.227	1.09 ± 0.26
Arabitol	0.827 ± 0.269	0.805 ± 0.210
Xylitol	2.18 ± 1.26	1.73 ± 0.65
1,5-Anhydrosorbitol	2.51 ± 0.51	2.26 ± 0.73
<i>myo</i> -Inositol	526 ± 127	$699 \pm 149^\dagger$

* NQ, not quantifiable.
 $^\dagger P < 0.005$.

Table 2 Polyol concentrations in mouse torso

Polyols	Diploid ($n = 14$) Mean $\pm$ S.D. (ng/mg)	Trisomy 16 ($n = 14$) Mean $\pm$ S.D. (ng/mg)
Mannitol	0.807 ± 0.135	0.807 ± 0.176
Sorbitol	5.71 ± 0.70	$4.53 \pm 1.05^*$
Galactitol	0.260 ± 0.025	0.245 ± 0.048
Ribitol	1.84 ± 0.29	1.73 ± 0.43
Arabitol	1.14 ± 0.13	1.08 ± 0.23
Xylitol	4.41 ± 0.18	4.11 ± 0.68
1,5-Anhydrosorbitol	5.39 ± 1.18	5.21 ± 1.19
<i>myo</i> -Inositol	229 ± 20	222 ± 52

* $P < 0.005$.

The concurrently measured polyol species also worked as internal controls for *myo*-inositol, which was the only polyol elevated in the brain. Thus, factors such as variability in tissue handling, sample preparation, chromatographic separation and mass spectrometric detection would have the same effect on these polyol internal controls as on *myo*-inositol. Also, short post-mortem delay does not cause appreciable breakdown of phosphatidylinositol, although polyphosphoinositides undergo hydrolysis under ischaemic conditions [7,8]. The one possible source of error in the determination of the free *myo*-inositol level would be if *myo*-inositol phosphates, released from inositol phospholipids, undergo rapid dephosphorylation. Because brains were excised from Ts16 and diploid mouse fetuses under the same conditions, it is reasonable to assume that any post-mortem effect on the free *myo*-inositol pool was the same in both groups of fetuses.

Quantitative data on *myo*-inositol and other polyols in torsos from Ts16 and diploid mice are shown in Table 2. The level of sorbitol (reduction product of glucose) was significantly reduced in the peripheral tissues of the Ts16 mouse. A reductive pathway, or possibly other pathways of glucose metabolism, may be affected in these fetuses. However, a generalized defect in carbohydrate metabolism was not evident, because other sugar metabolites were at normal levels. In DS, the level of plasma sorbitol was not altered [1].

Analysis of polyol species also indicated no change in the *myo*-inositol level of peripheral tissues, in contrast to brain tissues of the Ts16 mouse. In DS, plasma *myo*-inositol was not elevated [1]. Also, fibroblasts from DS subjects showed no increase [9]. Thus, tissue accumulation of *myo*-inositol in the Ts16 mouse and perhaps in DS is not systemic in nature. However, if the abnormality was localized to discrete organs, it might not be detected by whole-body analysis of the Ts16 mouse or by plasma analysis of DS subjects. It is also possible that peripheral organs are able to maintain *myo*-inositol homeostasis by efflux mechanisms, in spite of the increased transport. An increased cellular efflux for *myo*-inositol has been reported in DS fibroblasts [9]. A careful determination of the *myo*-inositol content of individual organs and their uptake and efflux of *myo*-inositol may resolve this quandary.

myo-Inositol homeostasis must be maintained in cells for the synthesis of membrane inositol phospholipids and related cellular signalling pathways. Millimolar levels within cells are achieved by active transport and by *de novo* synthesis. An augmented transporter or synthase activity may account for higher brain *myo*-inositol concentrations in the Ts16 mouse and in DS. Because its gene, SLC5A3, is localized to chromosome 21 [2], excess expression of the *myo*-inositol transporter may be responsible for the elevated brain *myo*-inositol. Differential ex-

pression of this transporter gene may account for varied tissue accumulation and may be verified by the *in situ* hybridization technique.

On the other hand, if the same transporter is expressed in every organ, the abnormal accumulation should also be detected in peripheral tissues. Thus, in addition to high-affinity transport, other factors may be involved in the elevation of brain *myo*-inositol. It is possible that in the Ts16 mouse and DS brains, an increased *myo*-inositol uptake at the blood-brain barrier is not countered by a similar increase in efflux. Additionally, over-expression of the SLC5A3 gene beyond the level predicted from simple gene dosage, involvement of a different *myo*-inositol transporter, altered *myo*-inositol synthesis or phosphatidyl-inositol turnover in brain are all possible mechanisms for altered *myo*-inositol homeostasis.

Our previous study on DS subjects [1] and the present data indicate that increased brain accumulation of *myo*-inositol is an abnormality common to DS and the Ts16 mouse. The common phenotypic abnormality further underscores the relevance of the Ts16 mouse as an animal model of DS. We would expect a transporter gene for *myo*-inositol, homologous to human SLC5A3 [2], to be present on mouse chromosome 16. Also, we have found an increased *myo*-inositol uptake by the Ts16 mouse cortical neurons (cultured) when compared with neurons from diploid animals (L. D. Acevedo, H. W. Holloway, S. I. Rapoport and H. U. Shetty, unpublished work), suggesting increased transporter-gene expression in these neurons.

The increased level of brain *myo*-inositol, apparently the consequence of a gene dosage effect, may have a pathophysiological significance in DS. Compared with adults, fetal tissues have several-fold higher concentrations of *myo*-inositol, and these levels vary during gestation [10,11]. The level of *myo*-inositol may be developmentally regulated by the transporter gene, SLC5A3. Increased *myo*-inositol uptake by fetal mouse brain cells, when compared with adult cells [12], supports the possibility of SLC5A3 gene involvement. The concomitant impaired *myo*-inositol homeostasis could possibly affect cell growth and differentiation.

In addition to its role in membrane structure and signal transduction, *myo*-inositol is a brain osmolyte whose level is modulated by the transporter during osmotic volume regulation [13]. Also, this transporter gene is an osmoregulatory gene [14]. Altered volume regulation and *myo*-inositol accumulation could

possibly affect cytoskeletal elements [15] and modulate the ion-channel gating in trisomy cells [4,16]. Additionally, the molecular bases for developmental abnormalities, such as anasarca in the Ts16 mouse [17] and nuchal oedema in the trisomy 21 fetus during the first trimester of gestation [18], have yet to be explained.

An aberration of one or more components of a signal-transduction pathway may give rise to multiple abnormalities. Thus, alteration of signal transduction pathways may serve as a guiding hypothesis for studying DS pathophysiology in molecular terms. Cellular signalling mediated by inositol phospholipids may be affected by increased uptake and accumulation of *myo*-inositol in this chromosomal disorder. Cultured neurons and other cell preparations from Ts16 mouse brain are appropriate materials in which to examine these molecular events.

We thank Dr. Stanley I. Rapoport, Chief, Laboratory of Neurosciences, for his expert advice and continued support, and Ms. Andrea Balbo for excellent technical assistance.

REFERENCES

- Shetty, H. U., Schapiro, M. B., Holloway, H. W. and Rapoport, S. I. (1995) *J. Clin. Invest.* **95**, 542–546
- Berry, G. T., Mallee, J. J., Kwon, H. M., Rim, J. S., Mulla, W. R., Muenke, M. and Spinner, N. B. (1995) *Genomics* **25**, 507–513
- Lacey-Casem, M. L. and Oster-Granite, M. L. (1994) *Crit. Rev. Neurobiol.* **8**, 293–322
- Rapoport, S. I. and Galdzicki, Z. (1994) *Dev. Brain Dysfunct.* **7**, 265–288
- Gropp, A., Kolbus, U. and Giers, D. (1975) *Cytogenet. Cell Genet.* **14**, 42–62
- Shetty, H. U., Holloway, H. W. and Rapoport, S. I. (1995) *Anal. Biochem.* **224**, 279–285
- Nishihara, M. and Keenan, R. W. (1985) *Biochim. Biophys. Acta* **835**, 415–418
- Ikedo, M., Yoshida, S., Busto, R., Santiso, M. and Ginsberg, M. D. (1986) *J. Neurochem.* **47**, 123–132
- Fruen, B. R. and Lester, B. R. (1991) *FEBS Lett.* **295**, 43–47
- Campling, J. D. and Nixon, D. A. (1954) *J. Physiol. (London)* **126**, 71–80
- Battaglia, F. C., Meschia, G., Blechner, J. N. and Barron, D. H. (1961) *Q. J. Exp. Physiol.* **46**, 188–193
- Fruen, B. R. and Lester, B. R. (1991) *Neurochem. Res.* **16**, 913–918
- Strange, K., Emma, F., Paredes, A. and Morrison, R. (1994) *Glia* **12**, 35–43
- Kwon, H. M., Yamauchi, A., Uchida, S., Preston, A. S., Garcia-Perez, A., Burg, M. B. and Handler, J. S. (1992) *J. Biol. Chem.* **267**, 6297–6301
- Cornet, M., Uhl, J. and Kolb, H.-A. (1993) *J. Membr. Biol.* **133**, 161–170
- Peng, S., Galdzicki, Z. and Rapoport, S. I. (1995) *Soc. Neurosci. Abstr.* **21**, 1713
- Miyabara, S., Gropp, A. and Winking, H. (1982) *Teratology* **25**, 369–380
- Szabo, J. and Gellen, J. (1990) *Lancet* **336**, 1133