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Inositol supplementation in pregnancies at risk of apparently folate-resistant neural tube defects

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To the editor

In 2005, Birth Defects Research (Part A) (BDRA) published a study on periconceptional dietary myo-inositol intake, finding no strong association with the risk of human Neural Tube Defects (NTDs) (Shaw, 2005). Now we write to bring new information on the use of inositol in families at risk of NTDs to the attention of the readers of BDRA.

NTDs are among the commonest birth defects, occurring in 0.5 to 2 per 1000 pregnancies. The neural tube is the embryonic structure that develops into the brain and spinal cord: the defects arise from failure of embryonic neural tube closure by the fourth week of pregnancy, causing malformations of the brain and spine (most commonly anencephaly and myelomeningocele). NTDs can be observed in association with chromosomal abnormalities, genetic diseases, teratogenic exposure, metabolic conditions. However, the majority of NTD cases are seen in isolation and should be regarded as multifactorial diseases. The MRC Vitamin Study (1991) found that 4 mg folic acid administration in the periconceptional period prevents approximately 70% of multifactorial NTDs. Further analysis has led to the idea that NTD risk is a function of the log(serum folate level) (Wald, 2001), suggesting that supplementation of folic acid dose levels above the 4-5 mg currently used for high risk pregnancies could prevent even more than 70% of NTDs. An alternative view, however, is that a proportion of human NTDs are intrinsically resistant to folic acid therapy, perhaps representing an NTD subtype with different etiology from cases that are prevented by folic acid. The finding of multiple NTD cases in a single family, despite high-dose folate intake (Cavalli and Copp, 2002) is consistent with this ‘folate-resistance’ hypothesis.

Further evidence of folate-resistant and folate-sensitive NTD subtypes has arisen through animal studies: NTDs in mice homozygous for mutations of the Pax3, Cart1, and crooked tail genes can be prevented by folic acid (Fleming et al, 1998; Carter et al, 1999; Barbera et al, 2002), whereas NTDs in the curly tail mutant mouse are resistant to folic acid (Greene, 1997).

There is no preventive therapy currently available for folate-resistant cases.
In an experimental model of folate-resistant NTD (the *curly-tail* mouse) both myo-inositol and D-chiro-inositol are effective in preventing NTD occurrence (Cogram, 2002).

It is suggested that exogenous myo-inositol enters the inositol phospholipid cycle and is incorporated into inositol phospholipids, including phosphatidylinositol diphosphate (PIP2). External signals, via cell membrane receptors, induce hydrolysis of PIP2, and generate diacylglycerol (DAG), which activates protein kinase C (PKC) isoforms. The subsequent phosphorylation of specific substrates results in the downstream correction of a cell proliferation defect that is responsible for spinal NTDs in *curly tail* embryos, thereby normalizing neural tube closure (Cogram, 2004).

The preventive effect of inositol on NTD occurrence is not restricted to *curly-tail* mice, since protection against diabetes-induced NTDs has been observed in other rodent models (Reece, 1997). Hence, the animal data support a distinct inositol-dependent metabolic pathway that, when stimulated, can prevent the cellular dysfunction leading to spinal NTDs.

In humans, lower inositol blood concentrations were found in pregnant women carrying NTD fetuses (Groenen, 2003), and periconceptional folic acid associated with inositol therapy has been linked to a normal livebirth, despite high NTD recurrence risk (Cavalli and Copp, 2002).

However, dietary inositol intake and NTD risk were not correlated statistically in a retrospective questionnaire analysis (Shaw, 2005). In a case of putative folate-resistant NTD, inositol supplementation was associated with a normal pregnancy outcome (Cavalli and Copp, 2002). These results suggest that inositol supplementation, but perhaps not normal dietary intake, could be beneficial in preventing folate-resistant NTDs in humans, as in rodents. Clearly, a scientifically rigorous test of this idea will require a randomized clinical trial.

Inositol is a six-carbon sugar alcohol found widely throughout mammalian tissues as phosphatidylinositol, and in cell membranes as phosphoinositide (Hasan, 1974). Inositol is an essential nutrient required by human cells in culture for growth and survival (Eagle, 1957). Moreover, its therapeutic use has been extensively studied in autism, and other psychiatric
disorders, in polycystic ovary syndrome, in patients on lithium therapy, and in respiratory distress syndrome (Benjamin, 1995; Colodny, 1998; Levine, 1997; Nestler, 1999; Howlett, 2003). No significant side effects of inositol therapy have been reported in any of the previous studies, even at high doses (up to 18 g/day in psychiatric patients) (Palatnik, 2001).

Here, we report on five successful pregnancies in three women with high NTD recurrence risk who received periconceptional supplementation with inositol and folic acid to prevent putative folate-resistant fetal NTDs.

Three Caucasian women from different parts of Italy, each with at least two previous NTD affected pregnancies, despite folate supplementation in at least one pregnancy, were referred to our clinic to discuss their NTD recurrence risk.

Two of the three women self-referred in two different pregnancies, giving a total of five pregnancies that received inositol supplementation.

After taking a personal and family medical history, involving pedigree drawing, chromosome analysis was always performed in both spouses (with normal findings in all cases). Glycemia was always found in the normal range in all the women.

A66G polymorphism in the methionine synthase reductase (MTRR) gene, as well as a 68 base pair insertion, the T2199C polymorphism in the cystathionine beta synthase (CBS) gene, and the C677T polymorphism in 5,10-methylene tetrahydrofolate reductase (MTHFR), previously shown to affect folate metabolism (Botto, 1998; Doolin, 2002; Relton, 2004) were studied in order to determine whether genotype might correlate with apparent genetic resistance to folic acid supplementation in these families. All women were informed that their previous NTD affected pregnancies could be folate-resistant and that a high recurrence risk of NTD (approximately 1 in 9 chance) (Seller, 1981) was to be expected in the next pregnancy.

After an extensive counseling procedure, all families took the personal and autonomous decision to undergo combined inositol and folic acid periconceptional treatment: 500 mg inositol and 5 mg folic acid daily, starting three months before conception and continuing until 60 days of pregnancy.
Pregnancies were followed by serial ultrasound examination at the 10th, 15th, and 20th weeks. Maternal serum alpha-feto-protein (AFP) blood levels were assessed at 15th-17th weeks.

Due to the recurrence risk of a genetic recessive disease, invasive prenatal diagnosis by chorionic villi sampling was performed in 1/5 pregnancies (PS).

Patients were particularly instructed to note any possible adverse pregnancy events, in particular myometral contractions, which might be associated with inositol therapy (Phaneuf, 1995).

Information of the course of each pregnancy, and the birth results were obtained by telephonic interview and/or via written medical reports.

The study was approved by an institutional Research Ethics Committee.

Characteristics of the five pregnancies and their outcomes are summarized in Table 1. Median maternal age was 32 years (range 29 – 38). Normal delivery was reported for 4/5 pregnancies. Cesarean delivery was reported in a single pregnancy, as one woman gave birth to a male baby affected by spinal muscular atrophy type I (SMA I, OMIM 253300). The baby was homozygous for a deletion of exons 7 and 8 of the SMN telomeric gene, and the parents proved to be heterozygous carriers on subsequent genetic analysis.

Genotyping results for MTHFR, MTRR and CBS are shown in table 2. Heterozygosity for the C677T polymorphism of MTHFR was present in one woman, while two women were heterozygous for the A66G polymorphism of MTRR. Patient PS was heterozygous at both loci. CBS genotyping revealed only wild type alleles. Importantly, none of the three women were homozygous for any polymorphic variant, the genotype that has been linked to NTD susceptibility in previous studies.

AFP blood levels were in the normal range, and ultrasound examination of all the pregnancies yielded normal findings. As expected from the prenatal monitoring, all the babies were born without any type of NTDs.

Moreover, all three women in their five pregnancies reported no side effects that could possibly be associated with inositol supplementation. While mild first-trimester uterine contractions were experienced in three out of five pregnancies (60%), no intense myometrial contractions, or other
adverse events, were reported in any of the pregnancies during or following inositol supplementation.

On the basis of the pregnancies studied in our series, therefore, we can find no evidence for adverse effects of inositol supplementation in the first trimester.

Mutations and polymorphisms in folate pathway genes have been intensively investigated and in some studies have shown an association with NTD risk (Shaw, 1998; Barber, 2000; Botto, 2000; Zhu, 2003). However their use in clinical practice to predict the occurrence risk for overall NTDs is likely to be impracticable, for the reason that even a significant increase in relative risk might be associated with a modest and non significant individual absolute risk.

Whether those maternal genetic polymorphisms could be considered useful to assess “folate-resistance” in NTD families, might be evaluated on different population and in greater series.

In conclusion, we have now documented three families, with a total of five pregnancies, in which there is a high risk of putatively folate-resistant NTDs, as indicated by the history of two NTD pregnancies in the presence of high dose folic acid supplementation. Inositol supplementation has been used in all five pregnancies with no evidence of intense myometral contractions or other adverse effects. All pregnancies have resulted in babies born at term, without NTDs. Taken together with the accumulating evidence of inositol prevention from experimental NTD models, we feel that our data indicate the need for a wider evaluation of inositol supplementation, in association with folic acid, in order to move towards a greater reduction in the overall frequency of NTDs.
Acknowledgements

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References


Table 1. Characteristics and outcomes of pregnancies at risk for putative folate-resistant NTDs

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<thead>
<tr>
<th>Case</th>
<th>Mat. age</th>
<th>Delivery week</th>
<th>Apgar score</th>
<th>Birth weight (g)</th>
<th>Sex</th>
<th>Delivery</th>
<th>Uterine contractions</th>
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<tr>
<td>DC*</td>
<td>29</td>
<td>40</td>
<td>9;10</td>
<td>3900</td>
<td>m</td>
<td>normal</td>
<td>mild (1st trimester)</td>
<td>None</td>
</tr>
<tr>
<td>DC</td>
<td>31</td>
<td>39</td>
<td>8;9</td>
<td>3350</td>
<td>f</td>
<td>normal</td>
<td>none</td>
<td>None</td>
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<tr>
<td>PS</td>
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<td>4;5</td>
<td>2400</td>
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<tr>
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<td>38</td>
<td>10;NA</td>
<td>3170</td>
<td>f</td>
<td>normal</td>
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<tr>
<td>SM</td>
<td>32</td>
<td>39</td>
<td>NA</td>
<td>3250</td>
<td>m</td>
<td>normal</td>
<td>mild (1st trimester)</td>
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§ Spinal Muscular Atrophy
* case previously reported (Cavalli & Copp, 2002)

NA: data not available
ND: not declared
Table 2. MTHFR, MTRR and CBS genetic polymorphisms in the mothers

<table>
<thead>
<tr>
<th>Mother</th>
<th>MTHFR C677T</th>
<th>MTRR A66G</th>
<th>CBS 68 bp ins</th>
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w = wild type allele
m = MTHFR: C677T allele; MTRR: A66G allele