Pyridoxine-Related Metabolite Concentrations in Normal and Down Syndrome Amniotic Fluid

Paddy Jim Baggot^{a, b} Anna Jane Y. Eliseo^b Nathaniel G. DeNicola^b
Jeremy A. Kalamarides^c James D. Shoemaker^d

^aHollywood Presbyterian Medical Center, Los Angeles, Calif., ^bSanta Teresita Medical Center, Duarte, Calif., ^cLake Erie College of Osteopathic Medicine, Erie, Pa., and ^dMetabolic Screening Laboratory, Saint Louis University School of Medicine, Saint Louis, Mo., USA

Key Words

Amniotic fluid • Down syndrome • Oxalate • Pyridoxine

Abstract

Introduction: Some studies of children with Down syndrome have found mild abnormalities in the metabolism of pyridoxine (vitamin B₆); therefore the present question is whether such abnormalities might also be present in the amniotic fluid of fetuses with Down syndrome. **Materials and Methods:** Archived specimens of amniotic fluid were obtained from chromosomally normal and from fetuses with Down syndrome. Gas chromatography/mass spectrometry quantitized B-related metabolites, including oxalate, xanthurenate, kynurenine and 4-pyridoxic acid. **Results:** Oxalate, a marker of pyridoxine deficiency, was elevated in the amniotic fluid of fetuses with Down syndrome. This result was statistically significant. The other marker results were not statistically significant. **Conclusion:** A marker of pyridoxine deficiency, oxalate is elevated in the amniotic fluid of fetuses with Down syndrome. These results in amniotic fluid are consistent with previous studies done in the urine of young children.

Copyright © 2008 S. Karger AG, Basel

Introduction

In the past, some have espoused vitamin supplementation regimes to treat postnatal Down syndrome. Most authorities consider these treatments unproven, if not disproven. In contrast, there is some literature on the biochemistry of pyridoxine in Down syndrome. The present study is intended to address the same biochemical questions in the fetus that were previously addressed in postnatal children.

As reviewed by Pueschel [1], a number of studies have been conducted on the biochemistry of pyridoxine-related metabolites and interactions with tryptophan metabolism in Down syndrome. Some are briefly reviewed here to address the clinical relevance of the present study. Coburn and Seidenberg [2] found reduced levels of pyridoxal-5-phosphate in Down syndrome leukocytes. In a double-blind study, Pueschel et al. [3] reported that 5-hydroxyindole levels were elevated after treatment with either 5-hydroxytryptophan or pyridoxine. Treatment with 5-hydroxytryptophan was not pursued because it was accompanied by infantile spasms in some cases [4]. In children with Down syndrome, the amplitude of corti-

cal auditory-evoked responses is high compared with normal children. Chronic treatment of 3-year-olds with pyridoxine appeared to decrease the cortical auditory-evoked potentials to the normal range [5, 6]. The relationship between pyridoxine and cortical auditory-evoked potentials was not stated. Pyridoxine (vitamin B₆) increased 5-hydroxytryptophan levels, confirming similar findings of earlier reports [5, 6].

McCoy et al. [7] conducted a series of experiments on children with Down syndrome involving a pyridoxine antagonist and tryptophan loading test. McCoy and his group measured oxalate, xanthurenate and kynurenine as markers of pyridoxine deficiency. They found levels of oxalate in children with Down syndrome to be higher compared with normal children; findings for xanthurenate and kynurenine were unconvincing. The hypothesis of the current study was that abnormalities of pyridoxine-related metabolites would be seen in the amniotic fluid from fetuses with Down syndrome when compared with fluid from normal fetuses. The present study attempts to extend McCoy et al.'s [7] work to amniotic fluid and thus confronts the question: does the same biochemistry apply to both fetuses and infants with Down syndrome?

Materials and Methods

Archived amniotic fluid specimens from normal fetuses and fetuses with Down syndrome were obtained from the cytogenetic laboratory at the Medical College of Virginia in Richmond, Va., USA. The abnormalities had been collected from 1993 to 1995, and the normals in 1996. Since old normal specimens had been discarded, normals from 1993 were not available. All specimens were stored in the same manner at -20°C, which is standard temperature for long-term freezing of metabolic specimens. Since this is well below the freezing point of water, oxidation of metabolites is unlikely. The median gestational age of the control specimens was 15.7 weeks and the median gestational age of the trisomic specimens was 16.7 weeks. This difference, while not zero, does not significantly affect the results.

Specimens were shipped on dry ice to Saint Louis University, Saint Louis, Mo., USA, for metabolic analysis. The samples were prepared and analyzed as previously described for urine [8]. All of the metabolites were derivatized with a trimethylsilyl-derivatizing agent, then analyzed by gas chromatography/mass spectrometry. Data (in µM) obtained from 22 Down syndrome and 41 normal specimens was analyzed parametrically, with means and standard errors. Simple t test was employed to compare the two groups where $p < 0.05$.

The protocol for this study was submitted to the institutional review boards at the Medical College of Virginia and Saint Louis University, both of whom waived further review since the work involved the use of archived specimens.

Table 1. Pyridoxine-related metabolites (µM) in Down syndrome and normal amniotic fluid

	Oxalate	4-Pyridoxic acid	Kynurenine	Xanthurenate
Down mean	36.477	1.084	1.180	0.005
Standard error	8.917	0.224	0.236	0.003
Normal mean	12.634	1.257	1.178	0.039
Standard error	3.311	0.282	0.259	0.034
p (pooled)	0.0038*	NS	NS	NS

* Statistically significant ($p \leq 0.05$).

Metabolites were analyzed by total ion current. As this is a first study in the pyridoxine biochemistry of fetal Down syndrome, it was not known which metabolites would be elevated, if any. Therefore the stable isotope dilution method with selective ion monitoring was not done. In future studies, it would be better to use selective ion monitoring, however, it would require isotopic markers for dilution.

Results

The level of oxalate was found to be nearly 3 times higher in Down syndrome amniotic fluid when compared to normal, and this result was statistically significant ($p = 0.0038$) (table 1). Levels of other pyridoxine-related metabolites, 4-pyridoxic acid, kynurenine and xanthurenate did not significantly differ between amniotic fluids from normal and from Down syndrome groups. Simple t test was employed to compare the two groups where p was less than 0.05.

Discussion

Some biochemical markers which can be used in pyridoxine deficiency include xanthurenic acid and kynurenine. These were not significantly different between normal and Down syndrome fetuses. 4-Pyridoxic acid is a marker of pyridoxine supplementation. Again there was no statistically significant difference. These findings would suggest that there was no classic deficiency of pyridoxine in fetuses with Down syndrome.

These results are consistent with the work of McCoy et al. [7] who required a pyridoxine antagonist and tryptophan loading in order to demonstrate differences with xanthurenate, kynurenine and 4-pyridoxic acid in chil-

dren. Use of pyridoxine antagonist and tryptophan loading are provocative techniques employed to detect a slight pyridoxine deficiency. McCoy et al. found that xanthurenate and kynurenine did not differ between normal children and children with Down syndrome unless provocative techniques were used. It is not surprising that no differences were found with these metabolites in our study, since no pyridoxine antagonist was used and no tryptophan loading tests were performed. The current results, concerning the B₆-related metabolites xanthurenate, kynurenine and 4-pyridoxic acid in amniotic fluid, are thus consistent with those of McCoy et al. [7].

While xanthurenate and kynurenine are considered the classic biochemical markers of B₆ deficiency, one could consider other metabolites as part of a broader realm of B₆-related metabolites. In the current data, statistically significant elevation of oxalate was found in fetuses with Down syndrome. Oxalate may behave as if it were a marker of pyridoxine (vitamin B₆) deficiency.

According to a mechanism proposed by Nath et al. [9], deficiency of pyridoxine impairs transaminase function, including glycine-glyoxylate transaminase, thereby increasing the glyoxylate pool. Oxalate elevation may result from pyridoxine deficiency, and oxalate falls with pyridoxine supplementation [9]. Oxalate is also clinically relevant as a component of calcium oxalate kidney stones. Therapy with either B₆ [10] or B₆ plus magnesium [11] may suppress oxalate and reduce the recurrence of kidney stones. While it may not be one of the two classical markers, oxalate indeed functions, as it were, as a marker of pyridoxine deficiency.

The oxalate results in table 1 are consistent with a subtle abnormality of pyridoxine metabolism in fetuses with Down syndrome compared with normal fetuses. It remains unclear why one marker of pyridoxine deficiency, oxalate, would be elevated. It is noteworthy that these results are consistent with the study by McCoy et al. [7] on the urine of infants.

This is the first paper to elucidate such a finding in fetuses. In a future effort, the use of stable isotope dilution techniques, marker metabolites and selective ion monitoring would allow more careful quantitative assessment of these metabolites. However, these techniques would also increase the difficulty and expense of the study.

There are other factors for consideration in future studies. Since oxalate is also known to come from dietary sources and from microbial metabolism, these might be confounding factors. Gestational age should also be considered. Metabolites in amniotic fluid tend to fall with advancing gestation. Gestational age could not explain

elevated oxalate in Down syndrome fetuses, as the gestational age in the Down syndrome group (16.7 weeks) was greater than the gestational age in the control group (15.7 weeks).

Conclusion

The methods described here would not be recommended for prenatal diagnosis. Prenatal diagnosis of in-born errors of metabolism is accurately done using a stable isotope dilution method, marker metabolites and selective ion monitoring. Prenatal diagnosis of chromosomal disorders is accurately done by chromosomal analysis and/or by fluorescence in situ hybridization. If the methods described in this paper were used for prenatal diagnosis, it is likely that some fetuses would be misdiagnosed. The goal of this study is to further our understanding of the pathophysiology and biochemistry of Down syndrome.

Over the past several decades, the health and longevity of patients with Down syndrome has improved. This improvement has not come from the discovery of a single magic bullet. Rather, it comes from the identification of many of the problems that these children have and their treatment. One example would be the testing of trisomic children for hypothyroidism and their treatment with thyroxine. It is hoped that with careful scientific study, greater understanding of fetal Down syndrome will lead to an improved quality of life for trisomic children.

Acknowledgments

This work was financially supported by the Michael Fund of the International Foundation for Genetic Research in Pittsburgh, Pa., USA. Cytogenetic analysis was done at Virginia Commonwealth University in Richmond, Va., USA. Editing assistance was provided by Zelma N. Wasserman.

References

- 1 Pueschel SM: Biomedical aspects of Down syndrome: biochemistry; Pueschel SM, Rynders JE (eds): Down Syndrome: Advances in Biomedicine and the Behavioral Sciences. Cambridge, Ware Press, 1982, pp 249–268.
- 2 Coburn SP, Seidenberg M: Leukocyte pyridoxal phosphate and alkaline phosphatase in Down syndrome and other retardates. *Am J Clin Nutr* 1969;22:1197–1203.
- 3 Pueschel SM, Reed RB, Cronk CE, Goldstein BI: 5-Hydroxytryptophan and pyridoxine. *Am J Dis Child* 1980;134:838–844.

- 4 Coleman M: Infantile spasms associated with 5-hydroxytryptophan administration in patients with Down syndrome. *Neurology* 1971;21:911-919.
- 5 Frager J, Barnet A, Weiss I, Coleman M: A double blind study of vitamin B₆ in Down's syndrome infants. Part 2. Cortical auditory evoked potentials. *J Ment Defic Res* 1985;29: 241-246.
- 6 Coleman M, Sobel S, Bahagavan HN, Coursin D, Marquardt A, Guay M, Hunt C: Double blind study of vitamin B₆ in Down's syndrome infants. Part 1. Clinical and biochemical results. *J Ment Defic Res* 1985;29: 233-240.
- 7 McCoy EE, Colombini C, Ebadi M: The metabolism of vitamin B₆ in Down syndrome. *Ann NY Acad Sci* 1969;166:116-125.
- 8 Shoemaker JD, Elliott WH: Automated screening of urine samples for carbohydrate, organic and amino acids after treatment with urease. *J Chromatogr Biomed App* 1991;562:125-138.
- 9 Nath R, Thind SK, Murthy MSR, Farooqui S, Gupta R, Koul HK: Role of pyridoxine in oxalate metabolism. *Ann NY Acad Sci* 1990; 585:274-284.
- 10 Curhan GC, Willett WC, Speizer FE, Stampfer MJ: Intake of vitamins B₆ and C and the risk of kidney stones in women. *J Am Soc Nephrol* 1999;10:840-845.
- 11 Gershoff SN, Prien EL: Effect of daily MgO and vitamin B₆ administration to patients with recurring calcium oxalate kidney stones. *Am J Clin Nutr* 1967;20:393-399.