Biotin-Related Metabolites In Normal And Down Syndrome Amniotic Fluid

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Citation

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Abstract

Objective: To analyze biotin-related metabolites, in order to see if a gene dosage mechanism is present for the enzyme holocarboxylase synthetase a gene on chromosome 21.
Methods: Amniotic fluid from fetuses with and without Down syndrome was analyzed by gas chromatography/mass spectrometrometry.
Results: Biotin-related metabolites (pyruvate, beta-lactate, hydroxyisovalerate and Leucine) were lower in Down syndrome. The ANOVA multivariate p value was 0.011. No evidence was found for deficiency of biotin or vitamin B¬12¬.
Conclusion: Data is consistent with gene dosage effect.

INTRODUCTION

Biotin (B_7) is a water soluble vitamin and acts as a cofactor for the biotin dependent carboxylases. These enzymes are associated in gluconeogenesis, fat and protein metabolism.[1] In a previous study, evidence has shown that pregnant mice with avidin-induced biotin deficiency produced litters with tetratogenic malformations.[2] Zempleni and Mock state that marginal biotin deficiency may also be teratogenic in human beings.[3] The association of fetal Down syndrome and biotin-related metabolites during pregnancy has been seldom investigated.

One of the pathophysiologic proposed mechanisms for Down syndrome (Trisomy 21) is a gene dosage effect.[4] In Down syndrome, there are three copies of each gene on chromosome 21, rather than two. A gene dosage mechanism would suggest that there would be a 50% increase in the protein products of those genes, which are on chromosome 21.[4]

These proteins may have metabolic effects if they are enzymes. In an inborn error of metabolism, a deficiency of an enzyme could result in an increase in the substrate for an enzyme and/or a reduction of the product of the enzyme. If a nutritional deficiency of biotin were present, one would similarly see an accumulation of substrates and/or a deficiency of products for those enzymes dependent on biotin. For a gene dosage effect in a chromosomal trisomy, one might look for the opposite: a reduction of the substrate or an increase of the product. The metabolites could then be examined to look for evidence of gene dosage effects.

One of the genes on Chromosome 21 (21q22.1) is for holocarboxylase synthetase (EC 6.3.4.10). [5] This gene codes for the enzyme that synthesizes holocarboxylase from biotin. Holocarboxylase is necessary for biotin-dependent enzymes, which have a carboxylase function. These enzymes insert carboxylate groups. If a gene dosage mechanism is present, any biotin-dependent enzyme could be affected by a 50% increase in holocarboxylase. This might result in a decrease of substrates and/or an increase in products.

One such enzyme is pyruvate carboxylase. [5] Pyruvate carboxylase metabolizes pyruvate to oxaloacetate. Thus, pyruvate could be affected. A second enzyme is 3-methylcrotonyl-CoA carboxylase.[5] This enzyme catalyzes an important step in the metabolism of the amino acid leucine.[6] Leucine is thus a marker for biotin deficiency. Further downstream from leucine is beta-methyl crotonyl CoA, which also requires the same enzyme (3-methyl-crotonyl-CoA carboxylase). A substrate marker for beta-methyl-crotonyl CoA is its glycine conjugate, 3- methylcrotonyl-glycine. Hydration of beta-methyl crotonyl CoA leads to beta- hydroxyisovalerate. Beta-hydroxyisovaleric acid is a sensitive marker for biotin deficiency.[7] A third enzyme is propionyl-CoA carboxylase, which carboxylates propionate to methylmalonate. Elevation of 3-hydroxypropionic acid (also known as beta lactate), is also a marker for biotin

deficiency.[7] Previous studies however, show that 3-hydroxypropionic acid is not the best indicator of biotin deficiency.[8] Any or all of these substrate metabolites (pyruvate, beta-lactate, leucine, and hydroxyisovalerate) might be elevated in biotin deficiency, or in holocarboxylase synthetase deficiency. Serum methylmalonate is a marker of cobalamin (vitamin B_{12}) deficiency and is not a biotin-related marker.

These three carboxylases are all dependent on holocarboxylase synthetase and biotin.[5] Since holocarboxylase synthetase is a gene which is located on Chromosome 21, it is reasonable to expect that a gene dosage effect might increase holocarboxylase activity. Were this to occur, one might look for a fall in metabolites, which are degraded by a carboxylase process. Our hypothesis was that pyruvate, leucine, 3-methylcrotonyl glycine or beta-lactate (3- hydroxypropionate) would be reduced in Down syndrome amniotic fluid when compared to normal amniotic fluid.

MATERIALS AND METHODS

At the time of the amniocentesis, the cells were centrifuged, the supernatant was frozen, and records of the samples were archived by the Cytogenetic Laboratory at the Medical College of Virginia. These were shipped on dry-ice over night to the Metabolic Screening Laboratory at Saint Louis University. Specimens were analyzed as previously described for urine. [9] The amniotic fluid supernatant samples were treated with urease and reacted with a trimethylsilyl derivatizing agent to prepare them for gas chromatography/mass spectrometry analysis. The specimens were injected into the gas chromatograph and then eluted over a period of 70 minutes.

Data from 22 Down syndrome specimens and 41 normal specimens were analyzed in the study. Analysis of variance was used for multivariate and univariate analysis of pyruvate, beta-lactate, beta-methyl-crotonyl glycine, hydroxyisovalerate and leucine. Methylmalonate was examined but not included with the Biotin-related markers, since it is a marker for vitamin B12 (cobalamin). A separate T-test was done for methylmalonate, a marker for cobalamin deficiency. All metabolites were normalized to creatinine to avoid concentration artifacts.

The protocol for this study was submitted to the Institutional Review Boards both at the Medical College of Virginia and Saint Louis University. Both boards waived further review due to the use of archived specimens.

? RESULTS

The concentrations of metabolites in amniotic fluid may vary with gestational age, and also with fluid shifts and hydration status. To avoid these artifacts, all metabolites values are divided by the standard, creatinine. The results were analyzed with analysis of variance. In all cases, the means for the biotin-dependent metabolites were less in Down syndrome than in normal specimens. Some univariate p-values were significant (beta- lactate and leucine); some were not (hydroxyisovalerate and methyl crotonyl glycine); and one was borderline (pyruvate). The multivariate p value was 0.011.

Methylmalonate was 25.435 +/- 18.302 in Down syndrome and 24.824 +/- 23.576 in normal specimens. The p value of this comparison was 0.915.

Table 1

Holocarboxylase/Biotin-related metabolites in Normal and Down Syndrome Amniotic Fluid

	Pyruvate/ Cr	B-Lactate/Cr	HO-Isoval/Cr	B-Me-Crot/Cr	Leu/Cr
Down	2.616	2.306	1.107	0.352	43.438
Normal	6.759	5.182	1.492	0.543	99.997
Down SE	1.717	1.12	0.321	0.314	14.354
Normal SE	1.286	0.839	0.24	0.235	10.751
Univar p value	0.058	0.044	0.34	0.628	0.002

All metabolite values divided by Creatinine (Cr) to prevent dilutional artifact; B-Lactate=Beta-lactate, also known as 3-hydroxypropionate; HO-Isoval = 3-hydroxyisovalerate, a standard biochemical marker for biotin; B-Me-Crot= Beta-Methyl Crotonyl Glycine; Leu=leucine

The univariate p values from analysis of variance are listed in the bottom row.

Multivariate p value= 0.011

DISCUSSION

The data on methylmalonate indicate no evidence of deficiency of vitamin B12 (cobalamin).

The results of this study suggest higher activity of holocarboxylase synthetase in fetuses with Down syndrome, which could be consistent with a gene dosage effect for holocarboxylase synthetase. The presence of holocarboxylase synthetase on Chromosome 21 may lead to an overproduction of enzymes, which depend on holocarboxylase synthetase for their function in fetuses with Down syndrome. The clinical effect could be similar to biotin excess.

Intestinal microbes can produce biotin, which results in

some variability in nutritional requirement.[10] Wolf et. al states the recommended daily intake is 35 micrograms for infants and 150-300 micrograms of biotin daily for adults.[11] The effectiveness of biotin may be reduced in biotinidase deficiency and inborn errors of metabolism involving biotin-related enzymes.[12] Biotinidase deficiency may be treated with biotin dosages of 5-20 mg daily in pediatric cases.[11] Since these dosages can be one hundred to one thousand times the Recommended Daily Allowance (RDA), it is suggested that biotin excess is safe. This data implies that enhanced activity of the biotin-related enzymes is unlikely to be harmful.

The results of the present study are consistent with a gene dosage effect for holocarboxylase synthetase. In several diseases, biotin has been safely used in dosages of more than 100 times the RDA. While a gene dosage effect is suggested, this gene dosage effect may not be harmful, as high doses of biotin seem to be harmless.

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