Hypothesis Generating Study Of Simple Carbohydrates In The Amniotic Fluid In Normal And Down Syndrome Pregnancies

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Citation

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Abstract

Introduction: Disorders of carbohydrate metabolism, in particular galactosemias, may be associated with mental retardation. For this reason, simple carbohydrates were investigated in the amniotic fluid from normal and Down syndrome fetuses.

Materials and Methods: Amniotic fluid specimens were obtained from a cytogenetic laboratory. These were archived specimens from prior amniocenteses. Gas-Liquid chromatography/mass spectrometry was used for the assays.

Results and Discussion: Gluconic acid was higher in Down syndrome than in normal amniotic fluid (p=0.039). This finding requires confirmation. Gluconate may be seen with therapeutic gluconates such as iron, zinc and calcium gluconate.

There was a trend (p = 0.07) toward elevation of dulcitol in the Down syndrome group. Dulcitol, also known as galactitol, is a toxic metabolite seen in galactosemia. This finding may benefit from further investigation with a larger sample size.

INTRODUCTION

In recent years there has been a growing interest in the biology and biochemistry of simple sugars. These are important in many biological systems and occur either in small molecules as monosaccharides and disaccharides, or in large polymers. In larger molecules, sugars may be combined with proteins to form glycoproteins, or mucopolysaccharides. Carbohydrates contribute part of the extracellular matrix and often may appear as cell surface antigens. Carbohydrates may be importantly involved in the development of the CNS [1].

Diseases affecting carbohydrates may also affect brain development and intelligence. Children who have galactosemia, for example, may have developmental delay, poor growth, speech delay, mental deficiency, and other problems. Other children with mucopolysaccharidoses may also have abnormal brain development [2]

As far as the authors are aware, relatively little has been done to investigate carbohydrates in Down syndrome. As part of a broader program to study the biochemistry of fetuses with Down syndrome, carbohydrates were studied from fetuses of normal and Down syndrome pregnancies. The goal was to generate hypotheses relating to carbohydrate metabolism in fetuses with Down syndrome.

MATERIALS AND METHODS

Amniotic fluid samples from normal and Down syndrome pregnancies were obtained from the Medical College of Virginia Cytogenetic Laboratory, Richmond, Virginia USA, and were then shipped on dry ice over night to the Metabolic Screening Laboratory at Saint Louis University, Saint Louis, Missouri, USA

Amniotic fluid was prepared and analyzed as Shoemaker and Elliott (1991) had previously described for urine [3].

Amniotic fluid samples were prepared for analysis with a derivatizing agent which insured that all small molecules would be volatile. Samples were then placed in the gas-liquid chromatography column for separation prior to subsequent quantization and identification. The amniotic fluid specimens were thus analyzed by gas-liquid chromatography/mass spectrometry.

The experimental protocol was submitted to Institutional Review Boards both at Saint Louis University, USA and the Medical College of Virginia, USA. Due to the use of archived specimens, both Review Boards waived further review.

Non-parametric analysis in the form of Mann Whitney rank sum test was performed because the data was not normally distributed. SigmaStat® 3.0 was used for the statistical analysis.

RESULTS AND DISCUSSION

Gluconic acid in Down syndrome (0.375 M/L) was higher than in normals (0.15 M/L) and the difference was statistically significant (p=0.039). This difference should be investigated further for confirmation. Gluconate is a counter-ion for nutritional supplements (e.g. calcium gluconate or ferrous gluconate). It is unclear why there should be a difference in amniotic fluid specimens between normal and Down syndrome fetuses. This finding could be a spurious result of testing a large group of metabolites.

There was a trend (p=0.07) toward higher dulcitol in the Down syndrome group. Previous animal studies indicated that dulcitol has toxic effects related to tissue glycogen metabolism [$_2$]. More recent studies show that dulcitol is a toxic metabolite found in galactosemia, an inborn error of galactose metabolism [$_4$]. While the difference was not statistically significant, the harmful nature of dulcitol suggests further investigation to determine whether it is evelated in Down syndrome amniotic fluid.

Figure 1

Table 1: Levels (uM/L) of carbohydrates in normal and Down syndrome amniotic fluid (Mann-Whitney Rank Sum Test)

Metabolite	*Down Median*	*Normal Median*	*P value**
Threitol	7.00	7.00	0.948
Erythritol	2.25	7.00	0.119
Arabinose	0.50	0.50	0.379
Fucose	0.10	0.50	0.261
Ribose	0.675	0.650	0.471
erythrit(2)xylose	8.25	8.00	0.629
fructose	1.50	1.50	0.197
glucose	11.75	12.50	0.965
galactose	679.25	1020.00	0.498
mannose	3.00	3.00	0.273
n-ac-glucosamine	0	0.100	0.306
lactose	0.50	0.50	0.137
maltose	0.50	0	0.173
xylitol	5.45	4.20	0.526
arabinitol	0.10	0.10	0.574
ribitol	0.10	0.00	0.112
allose	0.325	0.30	0.812
glucuronic acid	1.70	5.00	0.801
galactonic acid	9.00	8.00	0.920
gluconic acid	0.375	0.150	0.039
glucaric	0.05	0.00	0.379
mannitol	0.825	0.250	0.173
dulcitol	0.05	0.00	0.070
sorbitol	0.150	0.10	0.306
inositol	25.625	16.55	0.462
sucrose	0.00	0.00	0.994

*$p \leq 0.05$

CONCLUSION

In summary, the finding with statistical significance was that of elevated gluconic acid in amniotic fluid from fetuses with Down syndrome. There was also a trend toward dulcitol (galactitol) elevation. Both of these findings suggest testable hypotheses for future investigation into the disease mechanisms of Down syndrome.

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