POTENTIAL USE OF GLYCOSPHINGOLIPIDS OF HUMAN MECONIUM FOR BLOOD GROUP CHEMOTYPING OF SINGLE INDIVIDUALS

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1. Introduction

Since the discovery of blood group active substances, either glycoproteins [1–3] or glycolipids [4], in the human meconium, only limited information has been available regarding inter-individual variation in this tissue. The ABH blood group activity has been shown to correlate to that of the cord blood of the baby [5], but other blood group systems known to reside in glycoconjugates [6] have not been studied. The discovery of a large amount of blood group fucolipids of human meconium [4,7,8] has presented a source not only of fetal antigens [4] but of material for a chemical analysis (chemotyping) of blood group substances possible to correlate immunologically (serotyping) with blood, saliva or other products of the growing individual. Although most (80%) of the carbohydrate of meconium is glycoprotein [2] the glycolipids offer the advantage for chemical analysis in having 1 saccharide/molecule. Glycoproteins have several different oligosaccharides bound to each polypeptide [9,10]. Glycolipids of meconium may therefore for several purposes be preferred before glycoproteins of ovarial cyst fluid, the classical source for chemotyping of single individuals, see [11,12].

This communication briefly illustrates the chromatographic appearance of meconium glycolipids of single individuals and define structural methods for fingerprinting of fucolipids in a mixture.

2. Materials and methods

Meconia were collected and prepared as in [4]. All children except one were delivered at term by cesarean section and appeared healthy and normal at the physical examination after birth. Child 6 was delivered in week 36 (birth wt 1510 g) because of Toxicosis Gravidarum of the mother and was classified as small-for-dates but appeared otherwise healthy.

Total acid and non-acid glycosphingolipids were obtained after Soxhlet extraction, mild alkaline degradation, silicic acid and DEAE-cellulose chromatography [4,13]. Non-acid glycosphingolipids were further freed from contaminating alkali-stable phospholipids by silicic acid column chromatography after acetylation [14]. Subfractionation of non-acid glycosphingolipids of single individuals was done on silicic acid columns (Merck LiChroprep, particle size 15–25 μm) after acetylation using an increasing amount of methanol in chloroform as eluant.

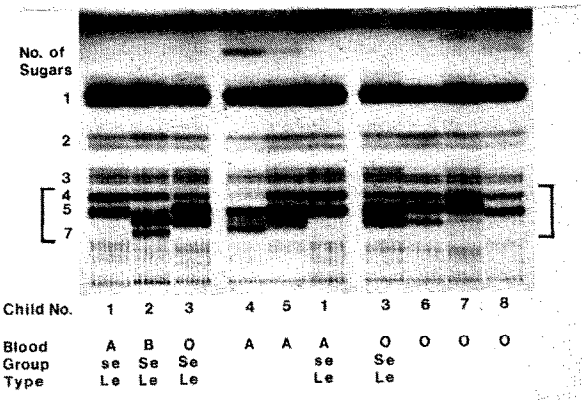

Fig.1. Thin-layer chromatogram of non-acid glycosphingolipids of meconia of 8 single individuals [1–8]. Figures to the left correspond to the number of sugars in the carbohydrate chains. The square brackets mark the interval of major blood group fucolipids subjected to immunological and chemical characterization. Blood group and secretor status of single individuals are indicated below the respective lane. Precoated HPTLC Si60 nanoplate (Merck) was used as layer, chloroform/methanol/water 60:35:8 (v/v/v) was used as solvent and the anisaldehyde reagent [23] for detection.

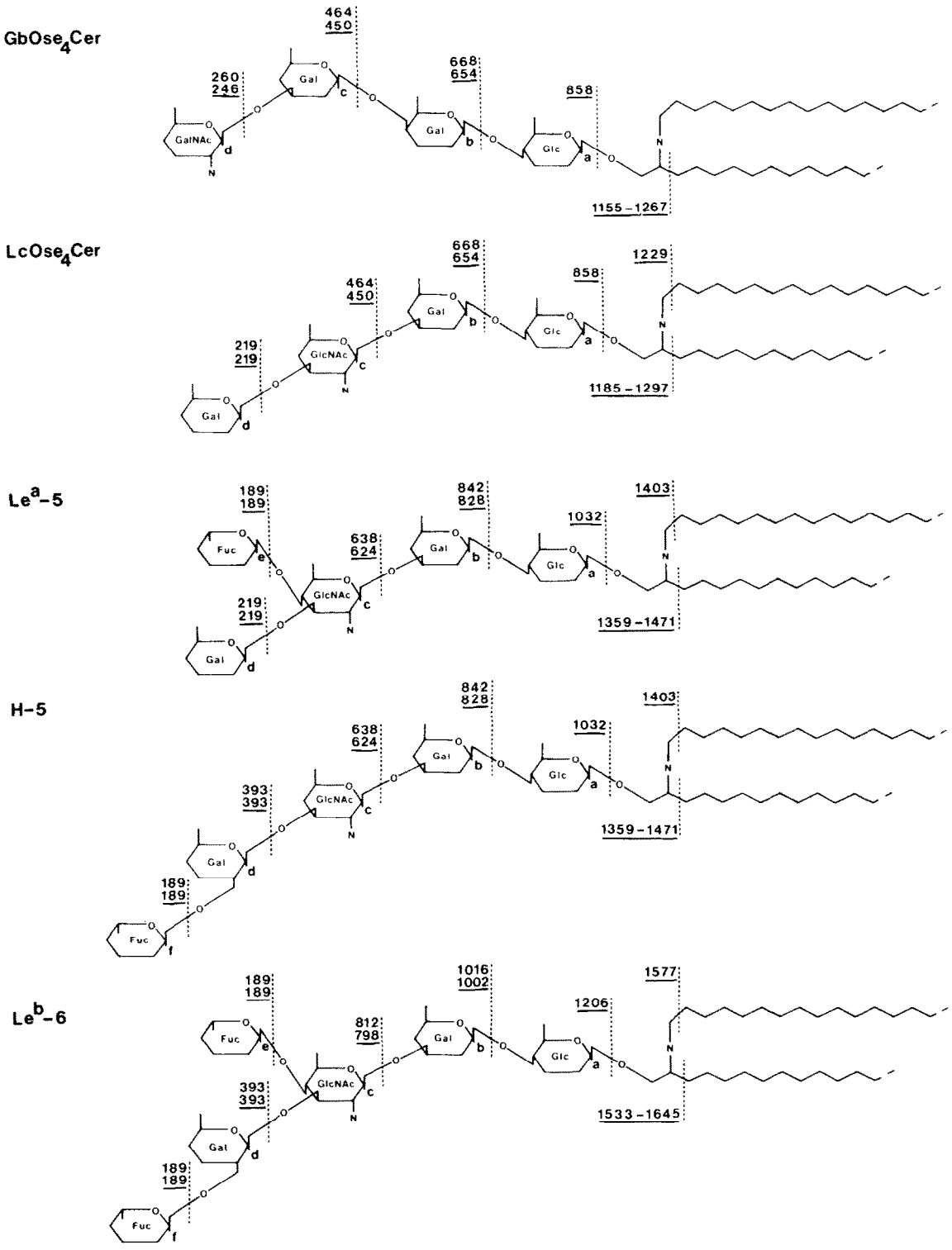

Fig.2. Simplified formulae of glycolipids concluded to exist in the 4–7-sugar interval of child 3 (fig.1). Figures not underlined give the weight of specific mass spectrometric ions of the permethylated derivatives, and figures underlined those of the permethylated-reduced derivatives (compare fig.3). Anomeric sugar protons for NMR spectroscopy are labelled alphabetically from the ceramide (compare fig.4).

For structural characterization permethylated [15] and $LiAlH_4$-reduced [16] permethylated derivatives were employed for both mass spectrometry and ^1H NMR spectroscopy. The mass spectrometer, an AEI MS 902 instrument, was equipped with a separate probe heater and for analyses of mixtures spectra were recorded continuously along a temperature-programmed distillation curve [17]. The 270 MHz proton NMR spectra were recorded on a Bruker WH-270 spectrometer operating in the Fourier transform mode, using C^2HCl_3 as solvent [7,18–20].

Blood group ABO typing of the children was done routinely on cord blood at the hospital. Lewis and secretor status was determined on 1 ml saliva ~2 years later using human anti-A, -B, -H, -Le^a and -Le^b antisera. Individual glycolipid fractions were tested for activity using liposomes in a haemagglutination-inhibition technique in the microscale [21].

3. Results and discussion

Total non-acid glycosphingolipids of meconium of 8 single individuals are shown in fig.1. The weight of the fractions varied from 85–225 mg. To enrich the major fucolipids the acetylated total fraction was separated by silicic acid column chromatography into 4 rather well defined subfractions. The third of these weighed 20–40 mg and contained tetra- to hepta-glycosylceramides corresponding to the deacetylated interval shown by brackets in fig.1. As evident this interval differed distinctly for individuals of separate blood groups. Weaker, more slow-moving bands also showed individual differences.

To illustrate the structural analysis some of the data of the 4–7-sugar interval of child 3 have been presented (fig.2–4). By mass spectrometry and NMR spectroscopy the glycolipids summarized in fig.2 were identified. This conclusion was supported by immunological analysis showing the presence of H, Le^a and Le^b blood group activity. The mass spectrum of the permethylated-reduced mixture (fig.3) presented ions for the complete saccharide and the fatty acid (sugar composition ions) as well as sequence ions. The masses of these are underlined at the formulae of fig.2. The spectrum of the non-reduced mixture (not shown) gave supporting sequence evidence (masses not underlined in fig.2). Supplementary information on binding configuration and in some cases binding position was provided by NMR analysis [7,19,20] as summarized by fig.4.

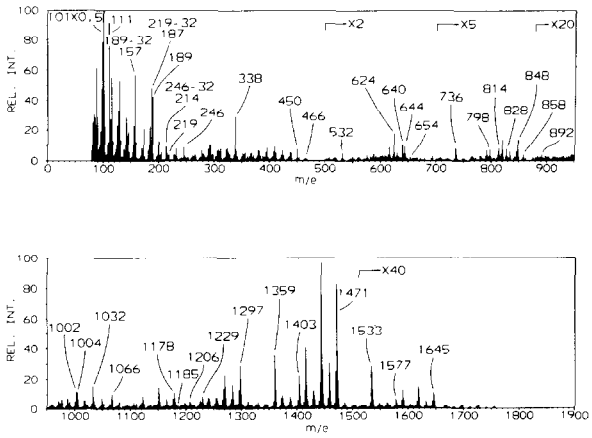

Fig.3. Mass spectrum of the permethylated-reduced derivatives of glycolipids of child 3 (interval within the square brackets of fig.1). For interpretation of specific ions, see fig.2. The conditions of analysis were: electron energy 23 eV, filament current 500 μA, acceleration voltage 6 kV, ion source temperature 285°C and probe temperature 245–290°C. The spectrum was recorded at the top of the total ionization curve (275°C).

Therefore, the meconium of child 3 (fig.1) had blood group H, Le^a and Le^b activity expressed primarily by glycolipids with 5, 5 and 6 sugars, respectively (fig.2). This was in line with the phenotype 0 Le(a–b+) secretor as found by saliva typing.

Analyzed in a similar way the 5–7-sugar intervals

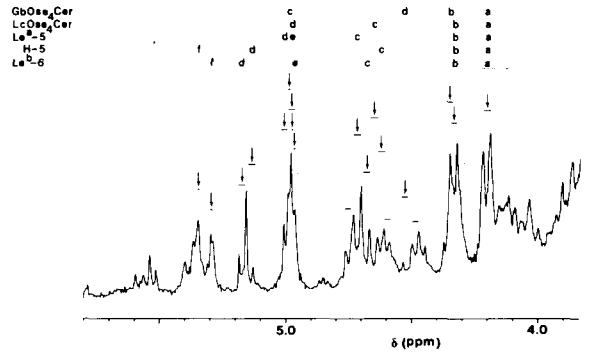

Fig.4. ^1H NMR spectrum (anomeric region) of the permethylated-reduced derivatives of glycolipids of child 3 (interval within the square brackets of fig.1). 3.0 mg in 0.45 ml C^2HCl_3, 3000 pulses at 40°C. Anomeric protons of the structures of fig.2 are indicated by their letters and an arrow above the corresponding signal. Coupling constants are marked by a horizontal line just below the corresponding arrow.

(fig.1) were shown to contain, for child 1 Le^a-5 (blood group A activity was lacking in this interval), and for child 2 H-5, Le^a-5, Le^b-6, B-6 and B-7 (the latter two formally obtained by addition of Galα1 → 3 to H-5 and Le^b-6, respectively). For children 4–8, not yet analyzed in detail, the slowest-moving band of this interval was probably A-7 (child 4), Le^b-6 (child 5 and child 6), H-5 (major band of child 7) and Le^a-5 (child 8). The fucolipids of this interval all appeared to be based on a type 1 chain (Galβ1 → 3GlcNAc).

Thus, based on these 4 criteria (thin-layer chromatography, mass spectrometry, NMR spectroscopy and immunology) and without the use of conventional degradative methods the identity of the major blood group glycosphingolipids in meconium of single individuals may be determined using ⩽3 mg of a mixture. Due to the large amounts of blood group fucolipids present in meconium this technique allows a chemical fingerprinting as shown here as well as a following isolation of single components. This is in contrast to erythrocytes and plasma from single individuals where only limited material is available for chemical studies [22]. Analysis of meconium may eventually prove to be an important supplement in attempts to correlate traditional blood group geno- and phenotype [6] with chemotype.

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