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Evidence for a correlation between ambient cholesterol levels and soluble plasma sialyltransferase enzyme activity

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While soluble forms of the sialyltransferase (sialyl-T) enzyme have been detected in significant quantities in serum, the exact source(s) of the enzyme, or the factors controlling its secretion are poorly understood. In this study, we have examined the relationship between ambient plasma cholesterol concentrations and sialyl-T activities and also levels of constituent plasma sialoglycoproteins (SGP). There was an inverse relationship between levels of the α2,6 sialyl-T enzyme and both total plasma cholesterol and HDL, although no such relationship was observed for the α2,3 enzyme. While there was no correlation between total cholesterol and the levels of plasma SGPs, there was an inverse relationship between the HDL component and α2,3 SGPs.

Keywords: cholesterol, sialyltransferase, sialoglycoprotein, Golgi, LDL, HDL

Introduction

The origins(s) of the soluble forms of sialyltransferase (sialyl-T) in the plasma have not been fully elucidated although it is likely that the liver, platelets and kidney are prime candidates as these tissues express particularly high level of the enzyme [1]. In addition, the release from liver cells of soluble forms of the α2,3 and α2,6 sialyl-T enzymes by a cathepsin D-like protease has been demonstrated in response to turpentine-induced inflammation [2, 3].

While the soluble form of the sialyl-T enzyme in plasma has been suggested to be involved in the acute phase inflammatory response [4], a precise physiological role for the enzyme in the soluble phase has not yet been identified. In order to catalyse the transfer of sialic acid, the enzyme must bind both the acceptor substrate and the donor sugar simultaneously and there is no evidence for sufficient levels of the activated sialic acid donor, CMP-NeuNAc, in the plasma to enable the enzyme to be fully functional.

Plasma enzyme levels may also be upset in certain disease states such as in cancer where there is an increase in sialy-T activity [5] and in Alzheimer's disease where we have previously demonstrated a significant decrease in enzyme activity [6, 7]. These changes may reflect altered enzyme activity at tissue level and an ultimate alteration in cellular protein glycosylation state [8, 9].

In order to gain a greater understanding of the function of soluble sialyl-T, it is important to investigate the factors controlling its activity. Previous studies have demonstrated that high serum cholesterol levels are associated with elevated protein sialic acid levels and that this may provide a possible mechanism underlying coronary heart disease [10]. In this study, we have investigated the relationship between ambient plasma cholesterol levels and the plasma activities of two sialyl-T enzymes which transfer sialic acid onto acceptor oligosaccharide chains in either α2,3- or α2,6-linkages.

Materials and methods

The subjects used in the study were drawn from both the general population and also from patients who had been referred to a lipid clinic at a major University Teaching Hospital (Ninewells Hospital, Dundee) due to high serum

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cholesterol levels. None of these patients were on cholesterol lowering therapy at the time of the trial and any subjects with specific disorders of lipid metabolism or other conditions likely to affect serum cholesterol levels (e.g. diabetes) were excluded from the study. All subjects were generally in good health and not on medication at the time of the study and the high plasma cholesterol levels in a subset of the subjects could generally be attributed to a high fat diet. The subjects ranged in age from 22 to 73 years (mean 47.7 years). Blood was collected from the patients by venepuncture into a sodium citrate tube. The samples were centrifuged at $15\,000 \times \mathbf{g}$ for 10 min to generate a platelet-poor plasma fraction. The samples were aliquotted into 1 ml fractions and stored at -20 °C until use, with lipid levels generally being measured within 48 h of plasma collection. The samples were obtained at the same time of day (early morning) in order to reduce the effect of diurnal variations and dietary factors on the parameters under examination. The protein content of the fractions was determined using the Folin phenol reagent [11].

The activities of the α2,3 and α2,6 sialyl-T enzymes were determined using a lectin-based microtitre plate assay system as previously described [7]. The transfer of sialic acid from the activated CMP-NeuNAc donor to the asialofetuin acceptor in α2,6 and α2,3 linkages was detected using the *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA) lectins respectively with boiled blanks serving as controls. The assays were standardized using fetuin.

Plasma sialoglycoproteins (SGP) levels were quantified using a lectin-based ELISA system using the MAA lectin to detect the Galα2, 3NeuNAc and the SNA lectin to detect the Galα2, 6NeuNAc and GalNAcα2,6NeuNAc disaccharide groups [7]. Fetuin also served as a reference in these assay systems. For the analysis of individual polypeptides, the plasma protein samples were separated by discontinuous SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes by electroblotting. The individual glycoproteins were detected on the blots using a DIG-glycan differentiation kit (Boehringer Mannheim) which employs lectins conjugated with the steroid hapten digoxigenin [8].

Plasma cholesterol and HDL levels were determined using the Technicon Axon enzymatic system which has an inter-assay coefficient of variation of 3%. Plasma LDL levels were determined directly using an LDL immunoseparation kit (Jenzyme Diagnostics). Relationships between different measurements were analysed by regression analysis.

Results

The subjects in the study had a wide range of plasma cholesterol levels (mean = 6.36 mmol l^{-1}, range = 2.73–10.12 mmol l^{-1}) and these levels generally increased with age (Fig. 1). This relationship was not due to either the LDL or HDL components which were not age-dependent (data not shown). There was a significant negative correlation between total cholesterol levels and the activity of the α2,6 sialyl-T enzyme (Fig. 2a) ($r = 0.693$, $p < 0.001$), although no such correlation was observed for the α2,3 enzyme (data not shown). There was also a relationship between α2,6 sialyl-T activity and the HDL cholesterol component (Fig. 2b) ($r = 0.457$, $p < 0.02$), but there was no correlation between plasma LDL levels and α2,6 sialyl-T activity. In addition, no relationship was detected between α2,3 sialyl-T activity and either HDL or LDL (data not shown).

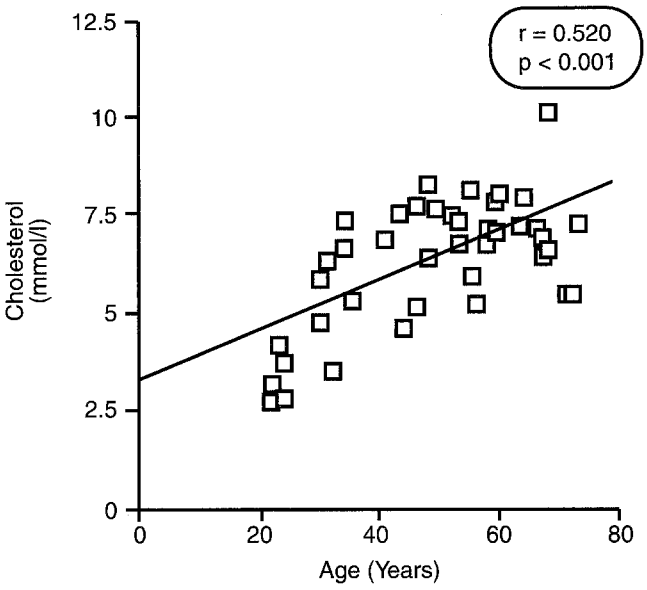

Figure 1. The relationship between age and plasma cholesterol levels as determined by regression analysis.

There was a negative correlation between HDL and plasma α2,3-linked SGP (Fig. 2c) levels although there was no relationship between the other lipid components and α2,3- or α2,6-linked SGP. Furthermore, there was no relationship between the enzyme activities and the plasma levels of the respective glycoprotein species (data not shown). In addition, although both α2,3 SGP and α2,6 sialyl-T were correlated with plasma HDL: levels, there was no significant correlation between these two variables ($r = 0.125$; $p = 0.535$).

The individual SGP constituents of selected plasma samples were analysed by lectin blot analysis. There were no obvious qualitative differences between the samples over the range of plasma cholesterol levels (Fig. 3) and when the combined intensities of the protein bands were plotted, there was no particular trend in the overall levels of SGP levels (data not shown).

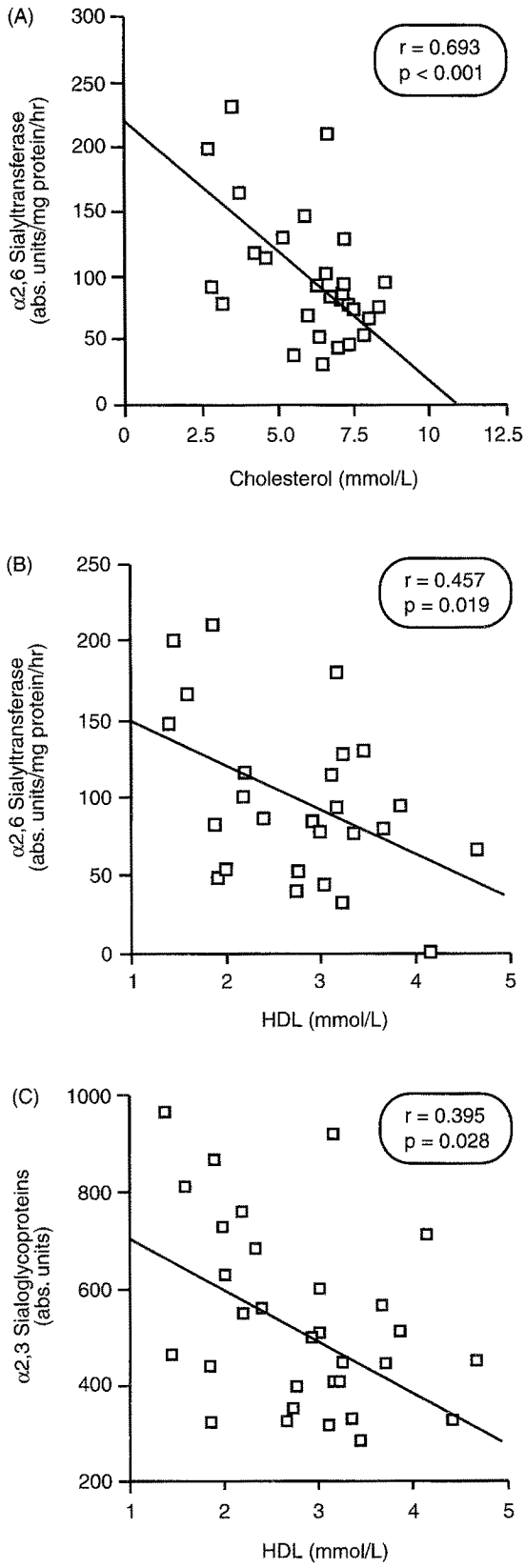

Figure 2. The relationship between (a) plasma cholesterol levels and α2,6 sialyltransferase activity; (b) HDL cholesterol and α2,6 sialyltransferase activity; and (c) HDL cholesterol and α2,3 sialoglycoprotein levels as determined by regression analysis.

Discussion

The origin and function of the soluble serum forms of sialyl-T remain to be elucidated. Previous studies have shown the enzyme to be released from liver Golgi membranes in vitro by the actions of cathepsin D [2, 3, 12] leading to the proposal that it may play a role as an acute phase reactant [4]. The release of a soluble form of the enzyme has also been proposed as a possible metabolic pathway with 12% of the membrane-bound enzyme being secreted [13]. The secreted form of sialyl-T retains its catalytic activity following secretion [4] suggesting a possible physiological role for the soluble enzyme, although the availability of the activated sialic acid donor, CMP-NeuNAc, in the plasma has not been demonstrated.

Expression of sialyl-T at the level of the Golgi can be influenced by a variety of factors including corticosteroids [14, 15], calmodulin antagonists [16], heavy metals [17], cytokines [18] and oncogenes [19] and in addition, plasma sialyl-T activity can also be stimulated by corticosteroids (Maguire and Breen, unpublished results). In this study, we have shown a possible correlation between soluble sialyl-T and ambient cholesterol levels. The fact that only the α2,6 sialyl-T enzyme is correlated with cholesterol levels may reflect the differential tissue distribution patterns of the two enzymes. The α2,6 sialyl-T enzyme is expressed primarily in the liver and kidney while the α2,3 enzyme is expressed at a much lower level in these tissues but is expressed at high levels in the colon, lung and brain [20]. This would suggest that the primary source of serum α2,6 sialyl-T may be the liver which also plays a major role in the synthesis and excretion of cholesterol within the body and may therefore be particularly susceptible to alterations in the ambient levels of cholesterol in the serum. The observed relationship between cholesterol and sialyl-T activity is further supported by the observation that the HDL component of the cholesterol appears also to correlate with serum enzyme activity. HDL is believed to be particularly involved in reverse cholesterol transport whereby cholesterol is transferred to the liver for excretion from the body in bile.

A relationship between serum SGP levels and ambient serum cholesterol levels has previously been suggested [10], and this has been proposed as a possible marker for coronary heart disease [21]. The latter study also demonstrated a correlation between serum SGP levels and 3 established risk markers for coronary heart disease (CHD) namely apolipoprotein B, daily cigarette consumption and leisure time physical activity [21]. However, an increase in serum sialic acid was only noted in the quartile of patients with the highest serum cholesterol levels. Indeed, a negative correlation was noted between HDL cholesterol and sialic acid. In the present study, we

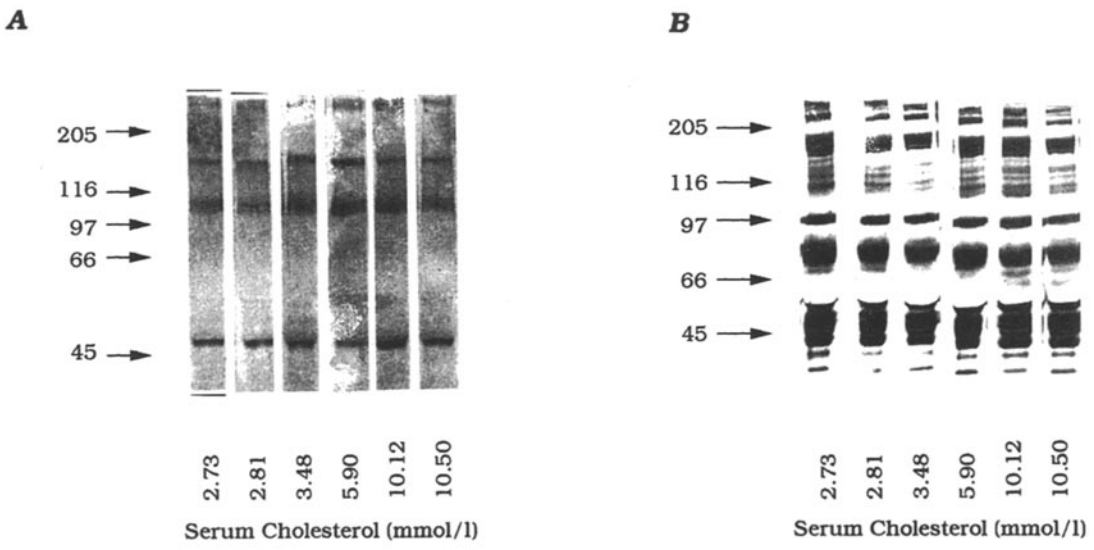

Figure 3. The profile of plasma (a) α2,3 and (b) α2,6 SGP levels as determined by lectin blot analysis. The migration of molecular weight standards are indicated.

have used a different detection system to quantify SGP levels in plasma, namely a lectin-based ELISA system which detected SGP levels expressing the Galα2,3NeuNAc, Galα2,6NeuNAc and GalNAcα2,6NeuNAc carbohydrate groups. In conjunction with lectin blot analysis of plasma protein samples from patients with a wide range of serum cholesterol levels, while we failed to detect any significant differences in the pattern of SGP expression or the staining intensities of individual protein bands with parallel changes in serum cholesterol levels, a negative correlation between α2,3 SGP levels and HDL cholesterol was observed, which is in good agreement with the previous results.

Although we have demonstrated a definite correlation between ambient plasma cholesterol levels and soluble sialyl-T activity, further studies are required in order to investigate whether there is a direct link between the two variables, and if changes in ambient cholesterol levels may elicit a significant effect on the subcellular distribution and release of sialyl-T.

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References

1. Wen DX, Svensson EC, Paulson JC (1992) *J Biol Chem* **267**: 2512–18.
2. Lammers G, Jamieson JC (1988) *Biochem J* **256**: 623–31.
3. Richardson K, Jamieson JC (1995) *Comp Biochem Physiol B-Biochemistry and Molecular Biology* **110**(2): 445–50.
4. Jamieson JC, McCaffrey G, Harder PG (1993) *Comp Biochem Physiol* **105B**: 29–33.
5. Dairaku K, Miyagi T, Wakui A, Tsuiki S (1983) *Gann* **74**: 656–62.
6. Maguire TM, Gillian AM, O'Mahony D, Coughlan CM, Dennihan A, Breen KC (1994) *Neurobiol of Aging* **15**: 99–102.
7. Maguire TM, O'Mahony D, Gillian AM, Dennihan A, Breen KC (1994) *Neurodegeneration* **3**: 129–33.
8. Gillian AM, Breen KC (1995) In Iqbal K, Mortimer JA, Winblad B, Wisniewski HM, eds *Research Advances in Alzheimer's Disease and Related Disorders*. pp. 429–36, New York: Wiley.
9. Maguire TM, Breen KC (1995) *Dementia* **6**: 185–90.
10. Wakabayashi I, Sakamoto K, Yoshimoto S, Masui H (1992) *Br Med J* **305**: 562–63.
11. Lowry GH, Rosenbourgh NJ, Farr AL, Randall RJ (1951) *J Biol Chem* **193**: 265–75.
12. Lammers G, Jamieson JC (1989) *Biochem J* **261**: 389–93.
13. Bosshart H, Berger EG (1992) *Eur J Biochem* **208**: 341–49.
14. Wang XC, O'Hanlon TP, Lau JTY (1989) *J Biol Chem* **264**: 1854–59.
15. Coughlan CM, Seckl JR, Fox DJ, Unsworth R, Breen KC (1996) *Glycobiology* **6**: 15–22.
16. Reboul P, George P, Geoffroy J, Louisot P, Broquet P (1992) *Bioc Biop Res Comm* **186**: 1575–81.
17. Hayes FD, Breen KC (1994) *Soc Neurosci Abstr* **20**: 1656.
18. Hanasaki K, Varki A, Stamenkovic I, Bevilacqua MP (1994) *J Biol Chem* **269**: 10637–43.
19. Le Marer N, Laudet V, Svensson EC, Cazlaris H, van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P (1992) *Glycobiology* **2**: 49–56.
20. Gillespie W, Kelm S, Paulson JC (1992) *J Biol Chem* **267**: 21004–10.
21. Lindberg G, Rastam L, Gullberg B, Lundblad A, Nillson-Ehle P, Harison BS (1993) *Artherosclerosis* **103**: 123–29.