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SIALIC ACID IN CARDIOVASCULAR DISEASES

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ABSTRACT

Sialic acid, the acylated derivatives of 9-carbon sugar neuraminic acid, present as terminal component of oligosaccharide chains of many glycoproteins and glycolipids, has been recognized to be involved in the regulation of a great variety of biological phenomena. Studies have shown that serum sialic acid predicts both coronary heart disease and stroke mortality and reflects the existence or activity of an atherosclerotic process. Most of the studies have shown an elevation in serum sialic acid concentration in coronary heart disease and a positive correlation between the raised serum sialic acid and the severity of the coronary lesions is observed. However, a few contradictory reports are also available. Racial differences in serum sialic acid have also been reported and correlated with international differences in the prevalence of atherosclerosis. Reduced sialic acid content of platelets, erythrocytes and lipoproteins may play important role in the pathogenesis of atherosclerosis. Elucidation of the mechanism of alternation in sialic acid concentration may throw more light on its potential clinical utility. Hence more studies are needed to designate sialic acid as a cardiovascular risk factor / marker.

KEY WORDS

Sialic Acid, Cardiovascular diseases

INTRODUCTION

Cardiovascular diseases are mainly caused by atherosclerosis and the pathophysiology of atherosclerosis is a complicated process governed by several risk factors. Enormous progress in our understanding this complicated process has been made in recent years and some new risk factors / markers have shown their relevance in this area.

Interest in the role played by sialic acid in the pathogenesis of atherosclerosis and as a predictor of cardiovascular events has rapidly increased in recent years especially since it has been shown to be a strong predictor of both coronary heart disease (CHD) and stroke mortality and reflector of the existence or activity of an atherosclerotic process (1, 2). Thereafter, many studies showed that serum sialic acid were elevated in cardiovascular disease (3 - 11). The sialic acid content of platelet, erythrocyte (RBC) and low density lipoprotein (LDL) has also been shown to play important role in the development of atherosclerotic complications (12, 13, 14). This review gives an account of the role played by sialic acid in cardiovascular diseases and highlights the areas where more studies are needed to establish the relevance of this biochemical parameter as a cardiovascular risk factor / marker.

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WHAT SIALIC ACID IS

Sialic acids comprise of N-or O-acyl derivatives of 9-carbon sugar neuraminic acid (5- amino- 3, 5-dideoxy-2 nonulosonic acid). Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids. In human beings it is present in body fluids (blood plasma, breast milk, gallbladder excretions, synovial fluid, sweat, gastric juices and urine) and tissues (erythrocytes, leucocytes, platelets, salivary glands, throat, stomach, cervix, colon, cartilage etc. (15). In blood plasma a large quantity of sialic acids are present in orosomucoid, fibrinogen, haptoglobin, ceruloplasmin, α_1-antitrypsin, complement proteins and transferrin (16, 17). It is also present as constituent of membrane glycoproteins of erythrocytes, leucocytes and platelets. About 80% of sialic acids in human serum is N-acetylneuraminic acid (Neu5Ac; NANA) and approximately 20% is Neu5Ac 9 Lt(18). Low amount of Neu 5, 9 Ac_2 have also been shown to be present in human blood serum (19). Similarly, in tissues the major sialic acid is Neu5Ac. The other small fractions are Neu5Ac_2En, Neu5Gc, Neu 5,7, 9Ac_3, Neu 5,8,9 Ac_3 etc, the role of which is

yet to be defined. Most of the research papers reported about NANA, the most abundant sialic acid.

The structure, occurrence and general functions of sialic acids have been extensively reviewed (20-25). Some important functions attributed to sialic acids are : (1) sialic acids contribute significantly to the overall negative charge of cell surface and glycoproteins. The negative charge contributes to cell to cell repulsion (antiadhesion effect), functioning stability and survival of glycoproteins in blood circulation and cell-to-matrix interactions; (2) due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks; (3) membrane sialic acids assist in cell - cell recognition and interaction and serving as chemical messengers in tissues and body fluids; (4) it serves as a component of cell surface receptors.

SERUM SIALIC ACID

Sporadic reports on serum sialic acid in cardiovascular diseases were published in 70s and 80s but the real interest in serum sialic acid in cardiovascular disease grew only after the report of Lindberg *et al*. (1) who showed that serum sialic acid is a strong predictor of cardiovascular mortality and may also reflect the existence or activity of an atherosclerotic process. Later on, many studies came up showing that serum sialic acids were elevated in cardiovascular diseases (3-10). Serum sialic acid levels correlated with carotid atherosclerosis, independently of major cardiovascular risk factors (26). It was also found to be raised in patients with NIDDM who were designated as a group with a markedly increased frequency of CHD, stroke and peripheral vascular diseases compared to non-diabetics (27). Serum sialic acid is considered as a marker of innate immunity and activated innate immunity is a risk factor for cardiovascular disease mortality in type 2 diabetes (28). Most recently, in a 17 year-follow up study serum sialic acid has been proposed to be a long - term predictor of CHD events in adults, especially in women (11).

Serum sialic acid has been correlated with serum lipids (29, 30). It was found to be higher in groups with high serum triglycerides or cholesterol and significantly lower in a group with high HDL cholesterol. Crook *et al*. (31) showed that serum sialic acid significantly correlated with systolic BP, fasting serum cholesterol and triglycerides and body mass index in females. In males serum total sialic acid significantly positively correlated with fasting serum cholesterol and triglycerides concentration and correlated inversely with hip/waist ratio. Masuda *et al*. (32) have shown that serum sialic acid reflects the status of blood glucose control and the progression of ischemic disease of the lower extremeties in NIDDM. Zahedi *et al*. (33) have found that it increased post prandially giving further insight as to why it is considered to be a cardiovascular risk factor. Serum sialic acid has shown positive correlations with blood platelet count, plasma fibrinogen, D-dimer, thrombin-antithrombin III complex and plasma alpha 2-plasmin inhibitor complex in type 2 diabetes (34).

Contradictory reports have been published on the possible relationship between serum sialic acid levels and the severity of coronary lesions in paitents with coronary heart disease (CHD). Allain *et al*. (3) found a positive correlation between raised serum sialic acid and the severity of the coronary lesions. Gokmen *et al*. (35) have found only lipid bound sialic acid to be related to the severity of coronary atherosclerosis.

In contrast, Salomone *et al*. (36) and Wu *et al*. (37) reported that serum sialic acid does not appear to be associated with the extent or severity of coronary artery disease (CAD) in patients with stable angina and suggested that the association with serum sialic acid and cardiovascular deaths described by others may reflect the role of mechanisms other than the severity of coronary artery narrowing.

The mechanistic aspect of the raised levels of sialic acid in cardiac patients is still not very clear though several possibilities have been suggested such as increase in acute phase reactants containing sialic acid (5-7, 16); increased sialylation of serum proteins (38) or reduction in desialylation of plasma glycoproteins (39). Gracheva *et al*. (9) have found enhanced activity of asialofetuin sialyltransferase of aorta intima in atherosclerosis as is the secretion of their soluble forms into patients plasma. The influence of sex hormones on the activity of sialyl-transferase and / or sialidase that control serum sialic acid remains to be investigated (11). Sialic acid was positively related to tumour necrosis factor (TNF α) and interleukin-6 (IL-6) and both these cytokines regulate C-reactive protein (CRP) and the synthesis of other acute-phase proteins (40). Increased concentration of CRP has been shown to be associated with an increased risk for coronary heart disease, sudden death and peripheral arterial disease and plasma sialic acid concentration has shown a strong correlation with C-reactive protein (37). Lindberg *et al*. (41) reported that mean serum level of glycoproteins were significantly higher in carotid atherosclerosis cases compared to control. However, when incorporated into the mathematical model, serum total sialic acid not only contributed additional information as to the risk of atherosclerosis, none of the three glycoproteins studied , contributed further once total sialic acid has been accounted for. Thus some other source of serum sialic acid or variations in the degree of sialylation of glycoproteins may be essential for understanding the relation between serum sialic acid and atherosclerosis. Gokmen *et al*. (42) have suggested that either the shedding or secreting of sialic acid from the cell or

cell membrane surface may be partly responsible for an increased serum sialic acid concentration following acute myocardial infarction. The correlation between serum total sialic acid and plasma fibrinogen may explain the association between elevated serum sialic acid and cardiovascular problems (5, 34). From these reports it seems that irrespective of the mechanism , serum sialic acid seems to be a causal risk factor and could prove useful in screening of population at cardiovascular risk. Recently, serum sialic acid has been shown to be a unique and novel marker that could be particularly useful in assessing myocardial cell damage in patients undergoing cardiac surgery (43).

The role of low density lipoprotein (LDL) in pathogenesis of CAD is well documented. The sialic acid content of LDL has been shown to play a very important role in this regard. The sialic acid content of LDL of patients with CHD was found to be several folds lower than those of healthy subjects (14, 44-46). A strong negative correlation has been established between the ability of LDL to stimulate intracellular lipid accumulation and their sialic acid content. Moreover, LDL from healthy subjects, desialylated by neuraminidase treatment induced the accumulation of intracellular cholesterol. These findings suggested that desialylation is an atherogenic LDL modification. Desialylation of LDL particles represents one of the first or the primary act of modification which is, apparently, a sufficient prerequisite for the development of atherogenic properties. Subsequent modifications just enhance the atherogenic potential of LDL (47). LDL in arterial wall atherosclerotic lesions was found to be sialic acid poor and ceramide enriched. These chemical changes promote LDL aggregation (48). Removal of sialic acid from the endothelial cell surface has been shown to increase the rate of receptor mediated endocytosis of LDL (49). Malmendier et al. (50) had observed that the removal of sialic acid from human LDL *in vivo* increased the rate of metabolic clearance. Similarly, Filipovic and Buddeck (51) found that removal of sialic acid residues accelerate the rate of internalization of the lipoprotein and subsequently the regulation of the metabolism of cellular cholesterol. In contrast, however, Melajarvi *et al*. (52) have shown that sialic acid poor LDL is catabolized more slowly than the sialic acid rich LDL. Contradictory reports on the role of LDL sialic acid content in atherogenesis are also available. Chappey *et al*. (53) showed that LDL sialic acid content is not a discriminant marker of early atherosclerosis in asymptomatic hypercholesterolemic subjects. Later on they (54) showed that LDL sialic acid content was increased in patients with both coronary stenosis and peripheral arterial atherosclerosis lesions compared with either no lesions or only one or the other type of lesions. The investigator explained this discordance with Orekhov and Co-workers, who showed that LDL sialic acid content in patients with advanced CAD was lower than that in healthy subjects, by the idea that desialylation may result from *in vitro* peroxidation of LDL due to the presence of EDTA in the buffer.

Racial differences in serum sialic acid have also been found. South Asian men have been shown to have higher serum sialic acid than the age-matched European men (55). Similarly, Caucasian living in Minneapolis had higher levels of serum sialic acid compared to Japanese living in Akita, Japan (56). These racial differences in sialic acid levels may reflect international differences in the prevalence of atherosclerosis (56). Thus serum sialic acid may be worth measuring in different racial groups and may be useful to assess individuals at risk of cardiovascular diseases and this requires large prospective studies (55).

Serum sialic acid levels are influenced by non-pathological factors like aging, pregnancy, smoking etc (15). Moreover, sialic acid is not specific for a given disease as variations in serum sialic acid levels have been found in various diseases such as cancer, diabetes, chronic renal failure, alcohol abuse, Salla's disease etc. (15).

PLATELET SIALIC ACID

The presence of sialic acid in human platelets had been demonstrated in early 60's but its function was not clear at that time. Later on, sialic acid's role in haemostatic function of the platelets was demonstrated in several studies. Platelet sialic acid has been shown to have the following functions: (i) It is responsible for the surface negative charge of the platelets which play an important role both in platelet adhesion and aggregation (57-60). An alteration in platelet sialic acid content results in altered electrophoretic mobility (61-64) (ii) platelet sialic acid is responsible for the life span of platelets as platelets depleted of sialic acid are rapidly cleared from circulation (65). Platelets from Bernard-Soulier patients which are deficient in glycoprotein I and sialic acid have a shortened survival time (66).

Coronary heart disease patients have been shown to have less platelet sialic acid content as compared to normal controls (12) and most of the platelet sialic acid was found to be susceptible to cleavage by neuraminidase, demonstrating sialic acid to be preferably localized at the outer surface. We have also found lower platelet sialic acid content in patients with acute myocardial infarction (AMI) as compared to normal controls (7). The lower platelet sialic acid could represent a contributing factor for higher aggregability of platelets in CHD patients. Further, lower sialic acid contents and the resulting lesser surface negative charge may cause less repulsion

between platelets and thereby facilitating aggregation.

Another possible causative role of low platelet sialic acid content is that sialic acid depleted platelets are rapidly cleared from the circulation. Platelet survival time theoretically measures the interaction of platelets with the arterial luminal surface. Thus the reduced platelet survival time could reflect increased platelet surface interaction due to several mechanisms in coronary atherosclerosis and an abnormal short platelet survival frequently occurs in patients with CAD (67-68).

The mechanism of these changes in platelet sialic acid is still not clear. Could it be due to increased serum sialidase activity (69) or could it be due to decreased sialylation of membrane proteins? A possible role of free radicals in desialylation of platelet proteins has also been suggested (70).

ERYTHROCYTE SIALIC ACID

Sialic acid is well known to be present in erythrocytes. Most of the sialic acid residues on the erythrocytes surface membrane are susceptible to cleavage by sialidase. The amount of sialic acid released enzymatically is correlated with the total sialic acid residues on the erythrocyte surface (71). The erythrocyte surface sialic acid has been shown to determine the life span of erythrocytes (72-75). It has been shown that desialylated erythrocytes are rapidly cleared from blood stream in rabbits (76) and man (77, 78). Another function attributed to RBCs sialic acid is that it provides negative surface charge to RBCs which is responsible for electrostatic repulsion between cells. It is hypothesized that by removing sialic acid residues and thus eliminating forces of mutual repulsion of similarly charged membranes, plasma sialidase activity agglomerates the red cell mass (79). An early decrease in erythrocyte sialic acid content has been suggested to influence the rheological properties of blood by increasing the adhesive energy of erythrocytes aggregates (80).

Reduced sialic acid content and electrophoretic mobility of erythrocytes has been observed in patients with AMI (10, 69, 81). It has been suggested that increased plasma sialidase activity in patients with AMI may be associated with clumps of desialylated erythrocytes that may alter flow in the microcirculation (82). The behaviour of sialidase present in the erythrocyte membrane could be more important in this regard. Venerando *et al*. (83) have shown difference in expression of two sialidase forms (acidic and neutral) in diabetics' erythrocytes. It has also been suggested that decreased erythrocyte sialic acid content may intensify the effect of fibrinogen on aggregation and disaggregation of erythrocytes and participate in the development of atherothrombotic complications (13).

CONCLUSION

Most of the studies have shown that serum sialic acid does play an important role in the pathogenesis of atherosclerosis and it could be useful in screening of population at cardiovascular risk. Serum sialic acid has been found to be elevated in cardiovascular diseases and positively correlated with the severity of the coronary lesions. However, a few contradictory reports have weaken this contention. Moreover, factors such as aging, pregnancy and smoking may affect sialic acid concentration and sialic acid is not specific for a given disease, thereby limiting its potential clinical usability (1). Serum sialic acid estimation, however, in different racial groups may be useful to assess individual at risk of cardiovascular diseases. Elucidation of the mechanism of alternations in sialic acid concentration may throw more light on its clinical utility. Platelet and RBC sialic acid contents could be important determinants as the cell surface sialic acid has a direct effect on cell surface charge, survival of cells in blood circulation and their aggregability, which play important role in the pathogenesis of a therosclerosis. More studies are, therefore, needed to designate sialic acid as a cardiovascular risk factor / marker.

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