The Possible Contribution of a General Glycosphingolipid Transporter, GM2 Activator Protein, to Atherosclerosis

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We previously found that oxidized low-density lipoprotein (LDL) elevated the expression of mRNA of GalNAcβ1-4[NeuNAcα2-3]Galβ1-4Glc-Cer (GM2) ganglioside activator protein, in human monocyte-derived macrophages. Recently, GM2 activator protein has become known as a general glycosphingolipid transporter as well as a specific cofactor for the hydrolysis of GM2 ganglioside by lysosomal β-hexosaminidase A. Accumulation of glycosphingolipids has been observed in the serum or aorta of atherosclerotic model animals and humans. The proliferation of aortic smooth muscle cells, elevation of LDL uptake by macrophages, interfering LDL clearance by the liver, and enhancement of platelet adhesion to collagen have been proposed as the underlying mechanisms of glycosphingolipid-mediated atherogenesis. The GM2 activator protein can bind, solubilize and transport a broad spectrum of lipid molecules, indicating that GM2 activator protein may function as a general intra- and inter-cellular lipid transport protein. Collectively, elevated levels of GM2 activator protein in the aorta may be another feature of human atherosclerosis.


Key words: Oxidized low-density lipoprotein, Glycosphingolipids, Atherogenesis, Macrophage

Introduction

Oxidized low-density lipoprotein (LDL) has been suggested to regulate various gene expressions in macrophages¹-³. To understand the effects of oxidized LDL on macrophage gene expression, macrophages incubated with native or oxidized LDL were analyzed by differential display technique. As a result of the study, we found the increased mRNA expression of GM2 ganglioside activator protein in macrophages incubated with oxidized LDL compared to those incubated with native LDL⁴. The GM2 activator protein acts as a specific cofactor for the hydrolysis of GM2 ganglioside by lysosomal β-hexosaminidase A⁵. Recently, this activator protein has been proposed to play a role in general glycosphingolipid transport⁶. Here we discuss the role of glycosphingolipids including gangliosides, and the possible contribution of GM2 activator protein in atherosclerosis.

Glycosphingolipid Accumulation in Atherosclerotic Lesions

Considerably higher glycosphingolipid levels in atherosclerotic lesions and serum have been observed in atherogenic model animals and humans. The concentrations of glucosylceramide (GlcCer), lactosylceramide (LacCer), GalNAcβ1-4Galβ1-4Glc-Cer (GA2), and ceramide trihexoside (CTH) were increased by approximately 7-fold in apolipoprotein E gene knockout mouse serum compared with controls, and N-glycolyl GM2 was also increased 3-fold⁷. Accumulation of neutral glycosphingolipids and gangliosides was also ob-
served in the aortic arch of the apolipoprotein E gene knockout mouse).

In the aorta of the Watanabe hereditable hyperlipidemic (WHHL) rabbit, an animal model for human familial hypercholesterolemia, neutral glycosphingolipids and gangliosides were increased by 5-fold and 12-fold, respectively, as compared with those in the aorta of normal rabbits. Likewise, GlcCer, LacCer, and GM3 ganglioside were increased by 13-, 12-, and 11-fold, respectively. Interestingly, sulfatide and NeuAc2-8NeuNAc2-3Galβ1-4Glc-Cer (GD3), inna-
tely absent in normal aorta, accumulated in great quan-
tities in the aorta of WHHL rabbits.

As a result of a quantitative comparative study of glycosphingolipid composition in the media and intima taken from normal lesions, fatty streaks, and atherosclerotic plaques of the human aorta, considerably accumulated glycosphingolipids have been observed in fatty streaks and atherosclerotic plaques as compared to normal lesions. The contents of GlcCer, LacCer, and GM3 ganglioside in plaques were 8-fold, 7-fold, and 12-fold higher than those in normal lesions, respectively. Other glycosphingolipids, Galβ1-3GalNAcβ1-4[NeuNAc2-3] Galβ1-4Glc-Cer (GM1), GD3, NeuAcα2-3Galβ1-3GalNAcβ1-4Gal[3-2αNeuAc] β1-4Glcβ1-1′Cer (GD1α), Galβ1-3Nacβ1-4Gal[3-2αNeuAc8-2αNeuAc]β1-4Glcβ1-1′Cer (GD1b), and NeuAcα2-3Galβ1-3GalNAcβ1-4Gal[3-2NeuAc8-2αNeuAc]β1-4Glcβ1-1′Cer (GT1b) were also identified in fatty streaks and plaques.

**Proposed Roles of Glycosphingolipids in Atherogenesis**

An outline of the reported proposed roles of glycosphingolipids in atherogenesis is shown in Table 1. Glycosphingolipids are now known to have a second-
messenger function in various cellular signaling path-
ways. LacCer has been suggested to stimulate the pro-
liferation of aortic smooth muscle cells (ASMC) (11). LacCer mediates the activation of NADPH oxidase that produces super oxide, subsequently stimulating the GTP loading of p21ras, activating the kinase cascade [mitogen-activated protein (MAP) kinase kinase, Raf, phosphorylated MAP kinase], and increasing c-fos expression. This signaling pathway has been suggested to be the underlying mechanism of LacCer-me-
diated proliferation of ASMC, “a hallmark in athero-
genesis”.

The addition of gangliosides has been reported to increase the uptake of LDL by mouse peritoneal mac-
rophages. Pretreatment of LDL with 5 nmol GM3 ganglioside resulted in a twofold increase in LDL up-
take and accumulation of cholesterol. The effect of GM3-treated LDL on the synthesis of cholesterol esters in macrophages was approximately seven times higher than in the presence of native LDL. These results indicate that ganglioside-rich LDL particles aggregate and are taken up by macrophages, consequently giving rise to fatty streaks. Furthermore, pretreat-
ment of LDL with gangliosides inhibits LDL binding to hepatocytes, suggesting that ganglioside-rich LDL may interfere with LDL clearance via the liver. Namely, ganglioside-rich LDL is presumably bound to scav-
enger receptors as well as oxidized LDL, but it remains to be elucidated. In addition, a recent study has reported that an enhanced expression of GM3 ganglioside in the human aorta was correlated with the pro-
cess of calcification. Collectively, gangliosides may contribute to the formation of fatty streaks and advanced atherosclerotic plaques. The effect of ganglio-
side-rich LDL has not been investigated in vivo, and should be studied in the future.

In a platelet adhesion study, GM3 and GD1 were reported to increase adhesion to collagen, mediated by the effect of gangliosides on collagen-binding integrin α2β1. This study suggests a mechanism for thrombus development at sites where gangliosides ac-
cumulate, and also indicates a possible role of ganglio-
sides in the process of atherosclerosis progression by a thromobogenic mechanism. The role of atherogenesis and the biological source of each glycosphingolipid is shown in Table 2. Accumulating data support the significant association between glycosphingolipids and atherosclerosis.

**Table 1. Proposed roles of glycosphingolipids in atherogenesis**

<table>
<thead>
<tr>
<th>Role</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferation of aortic smooth muscle cells</td>
<td>Enhancement of Integrin-mediated platelet adhesion to collagen</td>
</tr>
<tr>
<td>Elevation of LDL uptake and cholesterol accumulation by macrophage</td>
<td>Involvement in the process of aortic calcification</td>
</tr>
<tr>
<td>Inhibition of clearance of LDL via hepatic cells</td>
<td>Elevation of LDL uptake and cholesterol accumulation by macrophage</td>
</tr>
<tr>
<td>Involvement in the process of aortic calcification</td>
<td>Proliferation of aortic smooth muscle cells</td>
</tr>
</tbody>
</table>

**Association between LDL and Glycosphingolipids**

Glycosphingolipids do not exist in a free form in serum, and are not covalently bound but are associat-
ed to lipoproteins. Glycosphingolipids were not detected in lipoprotein-deficient serum, and elevated serum glycosphingolipid levels were observed in homo-
zygous familial hypercholesterolemic subjects. It seems that glycosphingolipids are associated with LDL.
in normal individuals as well as in patients with familial hypercholesterolemia and glycosphingolipidosis.\cite{21,23}

Oxidized LDL exerts time- and concentration-dependent stimulation on the activity of UDP-Gal: GlcCer,\(\beta\)-1,4galtransferase (GalT-2), which catalyzes the synthesis of LacCer, and on endogenous LacCer biosynthesis\cite{24,25}. By contrast, LDL inhibits LacCer synthesis in a time- and concentration-dependent manner\cite{26}. LDL specifically suppresses the activity of GalT-2\cite{27,29}. LDL binding, internalization, and degradation are essential for the LDL-mediated suppression of GalT-2 activity, suggesting an important role for the LDL receptors in this process\cite{29}. Modified LDL internalized by an LDL receptor-independent pathway, a scavenger receptor pathway, stimulates the activity of GalT-2, proposing that the entry of modified LDL into the cells by the scavenger receptors is likely to up-regulate rather than downregulate LacCer synthesis\cite{29}. This indicates the close relations between LDL and glycosphingolipid metabolism.

**Possible Contribution of GM2 Activator Protein to Atherogenesis**

Our study demonstrated that oxidized LDL up-regulates the mRNA expression of GM2 activator protein in human monocyte-derived macrophages\cite{4}. For hydrolytic conversion of ganglioside GM2 catalyzed by lysosomal \(\beta\)-hexosaminidase A, GM2 activator protein is required\cite{35}. A deficiency of GM2 activator protein results in the storage of GM2 ganglioside and severe neurological disease, Sandhoff disease\cite{3}. The GM2 activator protein is synthesized as a 22-kDa precursor bearing a single high mannose \(N\)-linked oligosaccharide chain on a peptide backbone of 18 kDa in the rough endoplasmic reticulum\cite{39}. In the Golgi apparatus, at least 70% of the 22-kDa precursor is converted to 24-kDa precursor by remodeling \(N\)-glycan to a complex-type oligosaccharide\cite{30}. About one-third of the precursor is secreted, and more than 90% of these secretory forms consist of the 24-kDa precursor\cite{30}. The intracellular remainder is segregated from the secretory pathway and processed in a post-Golgi compartment to yield a 20 kDa mature form, which exists in the lysosome\cite{30}.

GM2 activator protein contains at least three functional elements: a hydrophobic pocket, an oligosaccharide binding site, and an area that interacts with hexosaminidase A\cite{31-33}. A fluorescence dequenching assay specific for only the hydrophobic binding pocket demonstrated that various glycolipids inhibit the transport between liposomes of a self-quenching fluorescent lipid probe, octadecylrhodamine, by the activator protein, suggesting the binding of GM2 activator protein to glycosphingolipids\cite{34}. This result is supported by a study using thin layer chromatography overlay\cite{35}. Further, recent X-ray crystallographic studies of GM2 activator protein have revealed a large lipid binding pocket as the central overall feature of the structure with non-protein electron density within this pocket\cite{31-33}, therefore, GM2 activator protein is considered a lipid transfer protein\cite{6}. GM2 activator protein is a secretary protein as well as a lysosomal protein, and various cells have been reported to possess a carbohydrate-independent mechanism to re-capture the GM2 activator protein, with or without bound lipid, from the extracellular fluid\cite{6,36} (Fig. 1). Further, GM2 activator protein has been reported to bind, solubilize and transport a broad spectrum of lipid molecules, suggesting that GM2 activator protein functions as a general intra- and inter-cellular lipid transport protein\cite{6} (Fig. 1). Ganglioside concentration is remarkably low in isolated cells in contrast to the great number of gangliosides found in whole tissues in the aortic aorta, indicating that higher concentrations may accumulate in the intercellular space\cite{37}. However, it remains

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**Table 2.** The role in atherogenesis, biological source of each glycosphingolipid\textsuperscript{10-20}

<table>
<thead>
<tr>
<th>Glycosphingolipid</th>
<th>Role in atherogenesis</th>
<th>Biological source</th>
</tr>
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<tbody>
<tr>
<td>LacCer</td>
<td>sheer-induced increase in intercellular adhesion molecule-1 expression, proliferation of aortic smooth muscle cells</td>
<td>smooth muscle cells, monocytes, macrophages, platelets, endothelial cells, neutrophils, lymphocytes, fatty streaks, plaques</td>
</tr>
<tr>
<td>GlcCer</td>
<td>precursor of a potent proliferator of aortic smooth muscle cells, LacCer</td>
<td>fatty streaks, plaques</td>
</tr>
<tr>
<td>GM1</td>
<td>proliferation of aortic smooth muscle cells</td>
<td>fatty streaks, plaques</td>
</tr>
<tr>
<td>GM2</td>
<td>proliferation of aortic smooth muscle cells</td>
<td>fatty streaks, plaques</td>
</tr>
<tr>
<td>GM3</td>
<td>foam cell formation, calcification of aorta, platelet adhesion to collagen</td>
<td>fatty streaks, plaques</td>
</tr>
<tr>
<td>GD1</td>
<td>platelet adhesion to collagen</td>
<td>fatty streaks, plaques</td>
</tr>
<tr>
<td>GD3</td>
<td>proliferation and apoptosis of aortic smooth muscle cells</td>
<td>fatty streaks, plaques</td>
</tr>
</tbody>
</table>
unknown how such high concentrations of gangliosides accumulate in the intercellular space. In consideration of GM2 activator protein as a general intra- and inter-cellular lipid transporter, we speculate that this activator may be involved in intercellular glycosphingolipid accumulation. Although further studies are required to elaborate the relevance of GM2 activator protein to glycosphingolipid-mediated atherogenesis, our data suggest that oxidized LDL may upregulate GM2 activator protein, a general lipid transporter in macrophages, and subsequently enhance glycosphingolipid accumulation, which may be strongly associated with atherosclerosis (Fig. 2). Elevated levels of GM2 activator protein in the arterial wall may be another feature of human atherosclerosis.

Fig. 1. The function of GM2 activator protein as a general glycosphingolipid transfer protein.

GSL, glycosphingolipids; GM2-AP, GM2 activator protein; LacCer, lactosylceramide; GlcCer, glucosylceramide; r-ER, rough endoplasmic reticulum; MAPK, mitogen-activated protein kinase.

Fig. 2. A possible role of GM2 activator protein in atherogenesis.

References
13) Prokazova NV, Mikhailenko IA, and Bergelson LD: Ganglioside GM3 stimulates the uptake and processing of low density lipoproteins by macrophages. Biochem Biophys Res Commun, 1991; 177:582-587