Effect of lipoproteins on mesangial cell proliferation

YOJI NISHIDA, HIROAKI ODA, and NORIAKI YORIOKA

Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan

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Background. Triglyceride (TG)-rich lipoproteins have been reported to promote atherosclerosis, but little is known about their role in kidney disease or about their effects on mesangial cells. Accordingly, the purpose of this study was to investigate which lipoproteins could influence mesangial cell proliferation in vitro. We assessed the effect of various lipoproteins [very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low density lipoprotein (LDL), oxidized LDL, and high-density lipoprotein (HDL)] on the proliferation of cultured human mesangial cells and also assessed the influence of these lipoproteins on cytokine production.

Methods. We investigated the effect of various lipoproteins on cultured human mesangial cells using 3H-thymidine incorporation and cell counting assays and investigated the levels of several cytokines [interleukin (IL)-6, platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, and tumor necrosis factor-α] in mesangial cell culture supernatants after stimulation by the lipoproteins.

Results. Not only LDL but also TG-rich lipoproteins (VLDL and IDL) promoted the proliferation of mesangial cells up to certain concentrations, but cell growth was actually decreased at higher concentrations. Oxidized LDL caused a concentration-dependent decrease of 3H-thymidine incorporation, and HDL had no proliferative effect at any concentration. Exposure to VLDL, IDL, LDL, or a high concentration of HDL enhanced the secretion of IL-6, PDGF-AB, and TGF-β by mesangial cells, whereas TNF-α secretion was stimulated by oxidized LDL.

Conclusions. TG-rich lipoproteins, LDL, and oxidized LDL may be involved in mesangial cell proliferation and injury in patients with mesangial proliferative glomerulonephritis.

It has been long assumed that abnormalities of lipid metabolism influence the onset and progression of renal dysfunction [1]. Recently, low-density lipoprotein (LDL) has been found to be a growth factor for mesangial cells, and studies on abnormal lipid metabolism and renal dysfunction have been increasing [2]. It is also known that LDL cholesterol promotes atherosclerosis, and it has been reported that hypertriglyceridemia promotes atherosclerosis as well. Attention has been focused on intermediate-density lipoprotein (IDL) and remnant lipoprotein as factors promoting atherosclerosis. Similarities in the etiology of atherosclerosis and glomerulosclerosis have been noted [3], and it appears that IDL and remnant lipoprotein affect the glomeruli in the same way as the systemic blood vessels. Recent animal studies have suggested the possibility that very low density lipoprotein (VLDL) and IDL can cause glomerulosclerosis [4], but human studies have not yet been reported. In this study, we investigated the effects of various lipoproteins, including LDL and oxidized LDL, triglyceride (TG)-rich lipoproteins such as VLDL and IDL, and high-density lipoprotein (HDL), on cultured human mesangial cells.

METHODS

Cultured human mesangial cells were prepared by excising the intact renal cortex from kidneys removed because of renal carcinoma, isolating the renal glomeruli by sieving method, and passages the glomerular cells. After confluence was achieved, the cells were separated using trypsin-ethylenediaminetetraacetic acid (EDTA) and were passaged four to seven times in RPMI 1640 medium with 20% fetal calf serum (FCS). Lipoprotein-deficient serum (LPDS) was separated by the method of Goldstein, Basu, and Brown, and ultracentrifugation was used to separate VLDL (density < 1.006), IDL (d = 1.006 to 1.019), LDL (d = 1.019 to 1.063), and HDL (d = 1.063 to 1.215) [5]. After dialysis, the isolated lipoproteins were sterilized by filtration. Oxidized LDL was prepared by adding Cu2+ to LDL. The cultured human mesangial cells were uniformly distributed in microwells, which were incubated for three days in RPMI 1640 medium with 20% FCS, and then incubated for another 24 hours in RPMI 1640 medium with 0.5% FCS. When the cells approached quiescence, they were stimulated by adding LPDS or lipoproteins and were again incubated for 48 hours. At 16 hours before the completion of culture, [3H]-TdR was added at 1 μCi/well, and its incorporation was determined as an index of DNA synthesis. Counting of the number of cells in each well was done under a microscope at 48 hours after lipoprotein stimulation. The levels of cytokines [interleukin (IL)-6, IL-6, platelet-derived growth factor (PDGF)-AB, transforming growth factor (TGF)-β, and tumor necrosis factor (TNF)-α] in the culture supernatant were measured by

Key words: VLDL, IDL, LDL, HDL, cytokine, atherosclerosis, triglycerides.

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Table 1. Effect of various lipoproteins on the proliferation of cultured human mesangial cells measured by \(^{3}H\)-TdR incorporation

<table>
<thead>
<tr>
<th>Lipoprotein concentration (\mu g/ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>1,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>852.8 ± 181.7</td>
<td>1509.2 ± 39.6(^a)</td>
<td>1620.9 ± 378.7(^a)</td>
<td>1126.9 ± 362.0</td>
</tr>
<tr>
<td>IDL</td>
<td>820.2 ± 277.8</td>
<td>1413.2 ± 123.9(^a)</td>
<td>1269.1 ± 461.0(^a)</td>
<td>1050.8 ± 146.4</td>
</tr>
<tr>
<td>LDL</td>
<td>1208.1 ± 259.3</td>
<td>1817.8 ± 650.7(^a)</td>
<td>1525.6 ± 115.8(^a)</td>
<td>865.2 ± 101.6</td>
</tr>
<tr>
<td>ox-LDL</td>
<td>1315.2 ± 216.1</td>
<td>1083.4 ± 112.0</td>
<td>793.3 ± 110.3(^a)</td>
<td>361.0 ± 17.2(^a)</td>
</tr>
<tr>
<td>HDL</td>
<td>1235.2 ± 178.9</td>
<td>1286.4 ± 186.5</td>
<td>1637.8 ± 115.9</td>
<td>1520.0 ± 168.3 (cpm)</td>
</tr>
</tbody>
</table>

Results are the mean ± sd. Abbreviations are: VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; ox-LDL, oxidized LDL; HDL, high density lipoprotein.

\(^a\) \(P < 0.05\) vs. lipoprotein concentration = 0 \(\mu g/ml\), by repeated measures ANOVA plus the Bonferroni/Dunn post hoc test.

enzyme-linked immunosorbent assay (ELISA) at 48 hours after lipoprotein stimulation.

RESULTS

The addition of VLDL at concentrations of up to 100 \(\mu g/ml\), IDL of up to 50 \(\mu g/ml\), and LDL of up to 50 \(\mu g/ml\) induced the proliferation of cultured human mesangial cells, whereas cell growth was inhibited at higher concentrations. Oxidized LDL caused a concentration-dependent decrease of \(^{3}H\)-thymidine incorporation. HDL had no proliferative effect at any concentration (Table 1). Exposure to VLDL, IDL, LDL, or a high concentration of HDL enhanced the secretion of IL-6, PDGF-AB, and TGF-\(\beta\) by mesangial cells, whereas TNF-\(\alpha\) secretion was stimulated by oxidized LDL [6].

DISCUSSION

Not only LDL, but also TG-rich lipoproteins (VLDL and IDL) promoted the proliferation of mesangial cells up to certain concentrations, but cell growth was actually decreased at higher concentrations. Some investigators have assumed that this occurs because of oxidation during the preparation of the lipoprotein that results in cytotoxicity, but it was also shown that high lipoprotein concentrations promote apoptosis of mesangial cells [7]. Our lipoproteins showed almost no oxidation by the thiobarbituric acid reactive substances (TBARS) analysis, suggesting that promotion of apoptosis was the mechanism involved.

Mesangial cells have been reported to express VLDL and scavenger receptors [8], and it has been suggested that these receptors may be involved in the incorporation of VLDL and IDL. The lipoprotein concentrations used in this study did not reach 10% of those in normal human serum, but the lipoprotein concentration in the mesangial region appears to be lower than in normal serum because of its permeability. In healthy renal tissue, lipoproteins have no effect on mesangial cells, but glomerular impairment leads to an increase in the amount of lipoprotein in the mesangium, increased binding to the mesangial matrix, and increased lipoprotein incorporation by mesangial cells, and these changes are assumed to promote more renal impairment. Thus, lipoprotein does not appear to be a trigger for nephritis, but rather acts as an exacerbating factor.

In the case of oxidized LDL, inhibition of mesangial cell proliferation depends on the concentration of oxidized LDL added, and it appears possible that oxidized LDL has a cytotoxic effect on mesangial cells, is taken up by scavenger receptors, and causes secretion of various chemical mediators, leading to glomerulosclerosis [9].

In contrast, HDL does not appear to act directly on cells because no inhibition of cell proliferation was found at any concentration tested, although there have been reports that HDL promotes the proliferation of mesangial cells via tyrosine kinase [10].

Very low density lipoprotein, IDL, LDL, and oxidized LDL are all assumed to influence cytokine secretion from mesangial cells. It is not clear if changes in the proliferation of mesangial cells are a direct effect of lipoproteins or are mediated via cytokines, but it is clear that they regulate cytokine secretion from mesangial cells.

In conclusion, we found that LDL and TG-rich lipoproteins (VLDL and IDL) caused proliferation of mesangial cells, whereas oxidized LDL had a cytotoxic effect on mesangial cells. It is possible that these actions are mediated via cytokines such as IL-6, PDGF, TGF-\(\beta\), and TNF-\(\alpha\).

Reprint requests to Yoji Nishida, M.D., Second Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minamiiku, Hiroshima, 734-8551 Japan.

REFERENCES


