Oxidized low density lipoprotein stimulates aortic smooth muscle cell proliferation

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We have investigated the effects of oxidized low density lipoproteins (Ox-LDL) on aortic smooth muscle cell (SMC) proliferation and the biosynthesis of glycosphingolipids.

We found that Ox-LDL exerted a concentration, time, and temperature dependent alteration of cell proliferation and the biosynthesis of lactosylceramide. At low concentrations (5–10 µg/ml medium) Ox-LDL stimulated cell proliferation measured by an increase in the incorporation of [³H]-thymidine in cells and the synthesis of lactosylceramide, but not glucosylceramide synthesis. Oxidized LDL exerted a threefold increase in the incorporation of [³H]-galactose and [³H]-serine in lactosylceramide. The activity of lactosylceramide synthetase; UDP-galactose glucosylceramide β1 → 4 galactosyltransferase (GalT-2), but not glucosylceramide synthetase (GlcT-1) was stimulated by Ox-LDL. On the other hand, LDL suppressed the activity of GalT-2 in these cells. When cells were preincubated with antibody against Ox-LDL or GaIT-2 it compromised the oxidation LDL mediated stimulation in cell proliferation and GalT-2 activity. Similarly, D-PDMP an inhibitor of GalT-2 compromised the Ox-LDL mediated effects in cells. In contrast, L-PDMP further stimulated the Ox-LDL mediated cell proliferation and GaIT-2 activity. However, preincubation of cells with preimmune rabbit serum IgG failed to abrogate Ox-LDL mediated stimulation in cell proliferation and GaIT-2 activity.

In sum, we found that Ox-LDL stimulated aortic smooth muscle cell proliferation in culture. This effect resulted from Ox-LDL mediated activation of GaIT-2 that produced lactosylceramide. Lactosylceramide in turn, contributed to cell proliferation. Such correlations are supportive of the notion that GaIT-2 action mediates the signal transduction of Ox-LDL contributing to cell proliferation.

Key words: oxidized low density lipoproteins (Ox-LDL)/glucosylceramide (GlcCer)/lactosylceramide (LacCer)

Results

Effects of oxidized LDL and various antibodies on the incorporation of [³H]-thymidine in aortic smooth muscle cells

Oxidized LDL exerted a concentration-dependent stimulation of thymidine incorporation in cultured cells (Figure 1A). Maximum stimulation of the incorporation of thymidine into cells was observed with about 10–20 µg/ml Ox-LDL. This data indicate that at low concentrations oxidized LDL stimulates cell proliferation. In contrast, when cells were incubated with antibody against Ox-LDL, at various dilutions, it inhibited the proliferative effects in the cultured cells (Figure 1B). However, incubation of cells with antibody against lactosylceramide did not have any effects on the oxidized LDL mediated incorporation of [³H]-thymidine (Figure 1C). Finally, when the cells were incubated with antibody against GaIT-2, we observed a marked inhibition in the proliferation of Ox-LDL mediated proliferation in cells (Figure 1D). In sharp contrast, preincubation of cells with preimmune rabbit serum IgG did not abrogate the proliferative effects of Ox-LDL (Figure 1B–D, solid bars). These data indicate that oxidized LDL mediated proliferation of cells may involve the activation of the enzyme, GaIT-2 (see below).

Introduction

The role of Ox-LDL in atherosclerosis is becoming increasingly evident primarily because of the finding that such modified lipoproteins were found associated with the atherosclerotic plaques (Morton et al., 1986). Second, when Watanabe heritable hyperlipidemic rabbits were exposed to Probucol (a potent antioxidant) it prevented the atherosclerotic process (Carew et al., 1987; Kita et al., 1987).

Thus, although these lipoproteins could potentially cause injury to the aortic wall, very little is known regarding its biochemical mechanism of action. Our laboratory was the first to show that Ox-LDL stimulates the proliferation of aortic smooth muscle cells (Chatterjee, 1992). In a subsequent study, we reported that exogenously supplied lactosylceramide (LacCer) may stimulate the proliferation of smooth muscle cells (Chatterjee, 1991). Accordingly, we rationalized that one of the biochemical mechanisms in Ox-LDL mediated cell proliferation may involve activation of one or more of the metabolic enzymes in LacCer metabolism. Our studies reported here reveal that Ox-LDL specifically increases the activity of GalT-2, that in turn, produces LacCer and thus may contribute to the stimulation of aortic smooth muscle cell proliferation.

Effects of inhibitors and activators of GaIT-2 on oxidized LDL mediated stimulation in [³H]-thymidine incorporation in smooth muscle cells

As expected, incubation of cells with Ox-LDL (20 µg/ml) stimulated the incorporation of [³H]-thymidine into cells (Figure 2A,B, solid bars) compared to control 2A, B; open...
Fig. 1. Effects of Ox-LDL, anti-Ox-LDL antibody, anti-LacCer antibody, and anti-GalT-2 antibody on the incorporation of [3H]-thymidine in cultured aortic smooth muscle cells. Cells (×10^6) were seeded in 96 well flat bottom trays. After 6 days of growth, fresh medium containing Ham's F-10 medium was added to one set of cultured dishes and increasing concentrations of Ox-LDL antibody was added. Cells were preincubated for about 1 h with the antibody. Then, Ox-LDL was added to cells incubated ± Ox-LDL antibody. After incubation for 22 h, [3H]-thymidine (5 μCi/ml) was added to each well, incubation was continued for an additional 2 h. Subsequently, the incorporation of [3H]-thymidine into cells was measured as described previously (Chatterjee, 1992). Each experiment was done in triplicate, and the data is shown from six individual wells. (A) Effects of increasing concentrations of Ox-LDL (0–20 μg/ml) on the incorporation of [3H]-thymidine into cells. (B) Effects of anti-Ox-LDL antibody on the incorporation of [3H]-thymidine into cells. In this experiment, antibodies at various dilutions (1:50–1:200) was added to the cultured cells. Following incubation for 1 h, Ox-LDL (10 μg/ml) was added to each well and [3H]-thymidine incorporation measured as described above. (C) Effects of anti-LacCer antibody on the incorporation of [3H]-thymidine into cells. (D) Effects of anti-GalT-2 antibody on the incorporation of [3H]-thymidine into cells. The protocol was identical to that described in (B) above. The solid bars in B–D represent the effects of preimmune serum IgG plus Ox-LDL (20 μg/ml) on the incorporation of [3H]-thymidine in cells. The control values (100%) were 650 ± 53 × 10^3 c.p.m./mg protein.
Effects of incubation of cells with Ox-LDL on the incorporation of [3H]-galactose and [3H]-serine into various glycosphingolipids

When cells were incubated with small concentrations of Ox-LDL (5 and 10 µg/ml), there was a marked stimulation in the incorporation of [3H]-galactose into lactosylceramide. In contrast, Ox-LDL (5–10 µg/ml) did not alter the incorporation derived from [3H]-galactose radioactivity into glucosylceramide. At higher concentrations of Ox-LDL (25 µg and above) there was a marked decrease in the incorporation derived from [3H]-galactose radioactivity into glucosylceramide, compared to control. Interestingly, at low concentrations of Ox-LDL, we observed a threefold stimulation in the incorporation of [3H]-galactose into lactosylceramide in smooth muscle cells. Even up to 25 µg of Ox-LDL in the cultured medium there was a twofold higher incorporation of [3H]-galactose into lactosylceramide in these cells. At 100 µg/ml of Ox-LDL, the incorporation of [3H]-galactose into lactosylceramide in smooth muscle cells was moderately inhibited.

The incorporation of [3H]-serine into glucosylceramide was similarly minimally affected at low concentrations of Ox-LDL (5–25 µg/ml). It was however inhibited markedly at high concentrations (50–100 µg/ml) of Ox-LDL (Figure 3B). In contrast, the incorporation of [3H]-serine into lacto-
Fig. 3. Effects of Ox-LDL on the incorporation of [3H]-galactose and [3H]-serine into glucosylceramide and lactosylceramide in cultured aortic smooth muscle cells. Cells (×10⁵) were grown in six well plastic trays for 6 days in full medium containing fetal calf serum. On the sixth day, the medium was changed and cells were incubated with Ham's F-10 medium and increasing concentrations of Ox-LDL (0-100 μg/ml) and [3H]-galactose or [3H]-serine (5 μCi/ml). After incubation for 24 h at 37°C, the medium was removed and cells were harvested with PBS. Subsequently, cells were extracted with organic solvents, the glycolipids were purified by silicic acid chromatography and then, they were subjected to separation by HPTLC; chloroform methanol-water 65:25:4 (v/v) was used as solvent. Gel areas corresponding to standard GlcCer and LacCer were scraped and the incorporation of radioactivity was measured. Suitable aliquots of cells were also subjected to measurements of protein according to Lowry's procedure. Three such experiments were performed and the data of a representative experiment is shown here. (A) Incorporation of [3H]-galactose into glucosylceramide (○) and lactosylceramide (●). (B) Incorporation of [3H]-serine in glucosylceramide (○) and lactosylceramide (●).
Smooth muscle cell proliferation by oxidized LDL

Fig. 4. Effects of time of incubation with Ox-LDL on the activity of GalT-2. Cells were grown as described in Figure 3. Next, confluent cultures were incubated with Ox-LDL (10 μg/ml). At indicated intervals (0–8 hr) cells were harvested in cacodylate buffer (6.8). Subsequently, the activity of GalT-2 and GlcT-1 was measured as described in Materials and methods. GalT-2 activity (○), and GlcT-1 activity (△) (Havel et al., 1955). The results are average values of two experiments analyzed in duplicate. The activity of glycosyltransferases varied 5% among experiments.

GalT-2

GlcT-1

Time (hr)

0 1 2 4 8

0.5 1.0 1.5 2.0

Glycosyltransferase Activity (nmol/mg protein/3h)

slyceramide was stimulated in the presence of low concentrations of Ox-LDL (5–10 μg/ml) about 3.5-fold compared to control. As the concentration of Ox-LDL increased (50–100 μg/ml), there was a substantial decrease in the incorporation of [3H]-serine into lactosylceramide, but at all concentrations of Ox-LDL used in this study the incorporation of [3H]-serine into lactosylceramide was higher than control.

Effects of time of incubation with Ox-LDL on the activity of various glycosyltransferases and glycosidases

When cells were incubated with 10 μg of Ox-LDL/ml medium and the activity of GlcT-1 and GalT-2 was measured at various time points indicated in Figure 4, we found that Ox-LDL did not stimulate the activity of GlcT-1 enzyme activity. In contrast, within 2 h of incubation of Ox-LDL with cells, we found about a 75% increase in the activity of GalT-2. Cells incubated with Ox-LDL up to 4–8 h had only moderately higher activities of GalT-2 than control. Ox-LDL did not alter the activity of GlcT-2, and LacCer β1 → 4 galactosidase and LacCer β1 → 4 galactosidase (data not shown).

Effects of Ox-LDL and LDL concentration and temperature of incubation on the activity of GalT-2

Ox-LDL exerted a concentration-dependent stimulation in the activity of GalT-2 in these cells (Figure 5). In that, at low concentrations of Ox-LDL, we observed about a 40–60% stimulation in the activity of GalT-2. However, at high concentrations of Ox-LDL (100 μg/ml), the activity of GalT-2 was unaffected compared to control. Incubation of cells with LDL (Figure 5) did not stimulate the activity of GalT-2. In contrast, at high concentrations (25 μg/ml medium) it suppressed the activity of GalT-2 on the order of about 70% compared to control.

Incubation of cells at 4°C with Ox-LDL did not increase the activity of GalT-2. For example, the GalT-2 values obtained from cells incubated with and without Ox-LDL at 4°C were on the order of 1.06 nmol/mg protein/2 h and 1.05 nmol/mg protein/2 hr, respectively.

Effects of Ox-LDL, D-PDMP, and L-PDMP on the activity of GalT-2

As shown in Figure 6, D-PDMP exerted a concentration-dependent inhibition of the activity of GalT-2. In that, D-PDMP at 5 and 10 μM, decreased the activity of GalT-2 on the order of 50% and 40%, respectively, compared to control. Ox-LDL (10 μg/ml) stimulated the activity of GalT-2 on the order of 140%. When cells were incubated with Ox-LDL plus D-PDMP, it compromised the stimulatory effects of Ox-LDL in these cells. Incubation of cells with L-PDMP stimulated the activity of GalT-2. When Ox-LDL and L-PDMP (5 μM) were added together to cells, the effects were additive in regard to the stimulation of GalT-2 activity. The addition of Ox-LDL and higher amounts of L-PDMP (10–20 μM) sustained the stimulatory effects of GalT-2 activity in these cells (Figure 5).

Effects of various antibodies on the activity of GalT-2 in cultured aortic smooth muscle cells

In these series of experiments, cells were incubated with Ox-LDL in the presence and absence of various antibodies against Ox-LDL, lactosylceramide, and GalT-2 (Table 1).
Fig. 5. Effects of concentration of LDL and Ox-LDL on the activity of GalT-2 in aortic smooth muscle cells. The protocol of this experiment was identical to the one described for Figure 4 except cells were incubated with increasing concentrations of LDL (O) or Ox-LDL (●) for 2 hr. Subsequently, the activity of GalT-2 was measured as described in Materials and methods. Two such experiments were performed and the average values are presented. The activity of GalT-2 varied 5% among experiments.

Fig. 6. Effects of D-PDMP, L-PDMP, and Ox-LDL on the activity of GalT-2 in aortic smooth muscle cells. Cells were incubated with increasing concentrations of D-PDMP, L-PDMP (5–20 μM) for 1 h at 37°C. Next, Ox-LDL (10 μg protein/ml) was added and incubation was continued for an additional 2 hr. Subsequently, medium was removed and the cells were harvested in cacodylate buffer and GalT-2 activity was measured as described in Materials and methods. The control values for GalT-2 (100%) were 2.75 nmol 12 h/mg protein.

We found that whereas Ox-LDL stimulated the activity of the enzyme, this effect was compromised when cells were preincubated with antibody against Ox-LDL. However, antibody against Ox-LDL itself did not have any effects on GalT-2. When cells were incubated with antibody against lactosylceramide and Ox-LDL we found a significant stimulation in the activity of GalT-2. In contrast, antibody against GalT-2 by itself decreased the activity of GalT-2 in these cells on the order of about 50% compared to control. This antibody when added along with Ox-LDL compromised the effects of Ox-LDL in stimulating the activity of GalT-2.
together, these findings imply that Ox-LDL may be taken

effect on the activity of GalT-2 in cell-free extracts. Taken
activity of GalT-2. Moreover, Ox-LDL did not impart any
scribed by Steinberg and co-workers (unpublished obser-
7) may involve the binding of Ox-LDL to a putative cell
vations). This tenet is also supported with the evidence
Ox-LDL at low concentrations can stimulate the prolifera-
sylceramide are enriched in the atherosclerotic plaque in
LDL mediated proliferation of aortic smooth muscle cells.

These findings are of great biological and
clinical significance as Ox-LDL has been implicated in the
clinical significance as Ox-LDL has been implicated in the
process of cell proliferation. In that, antibodies against Ox-LDL
blocked the proliferation process, and inhibitors of gluco-
sylceramide synthesis, lactosylceramide synthesis as well as
the GalT-2 antibody directly inhibited both the cell
proliferation as well as the activity of GalT-2. How anti-
body against GalT-2 impairs Ox-LDL mediated induction in
cell proliferation is not understood from our studies.
Particularly in view of the belief that most, if not all, GalT-
2 is localized in the Golgi apparatus. This would imply that
GalT-2 antibody would have to have access to the enzyme
and therefore, must enter the cell. Alternatively, if that
pool of GalT-2 involved in the signaling pathway is present on
the cell surface, then the anti-GalT-2 antibody may have access and then, compromise the effects of Ox-LDL.
In this regard, we have preliminary evidence that a signifi-
cant amount of GalT-2 activity is present on the cell sur-
face. Studies are in progress to elaborate this phenom-
ena, further.

Since smooth muscle cells do not have detectable
amounts of galactosylceramide, we infer that radioactivity
derived from [3H]-galactose is incorporated into glucosy-
lceramide, and subsequently into lactosylceramide as a con-
sequence of stimulation of GalT-2 antibody by Ox-LDL.
Activators of GalT-2 such as L-PDMP stimulated both cell
proliferation as well as the activity of GalT-2. Whether or
not this reflects an additive increase in LacCer mass in
cells is not known. Previously, Radin and co-workers
showed that D-PDMP exerted a marked effect on cell prolifera-
tion in vitro and in vivo (Chatterjee et al., 1986; Radin et al., 1993). Moreover, Inokuchi showed that
L-PDMP stimulated the activity of GalT-2 in B-16 melano-
ma cells (Inokuchi, J.I., personal communication) Taken
together, our data indicate that the activation of GalT-2
mediates the signal transduction process involved in Ox-
LDL mediated proliferation of the aortic smooth muscle
cells. We found that Ox-LDL mediated stimulation in the
activity of GalT-2 ranged between 40–75% higher than
control. This may be due batch to batch variation in Ox-
LDL, prepared from the LDL from different normalip-
demic volunteers. In addition, this difference may be due
to animal to animal variations as the primary cultures of
aortic smooth muscle cells were derived from different
rabbits. Nevertheless, in all of our experiments pursued
over the last few years, Ox-LDL consistently stimulated the

Recently, an epoxide derivative of glucosylceramide has
been shown to specifically inhibit the endogenous synthesis
of lactosylceramide (Zacharias et al., 1994). Availability of
such an inhibitor may be helpful in directly assessing the
role of GalT-2 in the Ox-LDL mediated cell proliferation.
Our laboratory is currently involved in the molecular cloning
of the enzyme GalT-2. The availability of suitable

Discussion
This study led to several significant findings. First, we found
that Ox-LDL mediated stimulation of cell proliferation
was compromised by the antibody against Ox-LDL or the
antibody against the enzyme GalT-2. Second, Ox-LDL stimulated the endogenous synthesis of lactosylceramide, but not glucosylceramide from exogenously added radioactive precursors. Third, Ox-LDL specifically stimulated the activity of GalT-2 and this effect was time, concentration and temperature dependent. Fourth, Ox-LDL mediated stimulation of the activity of GalT-2 was compromised by an inhibitor of GalT-2, i.e., D-PDMP, and was stimulated by an activator of GalT-2, i.e., L-PDMP. Finally, the Ox-LDL mediated stimulation of activity of GalT-2 was compromised by antibody against GalT-2 and Ox-LDL itself, but not by antibody against lactosylceramide.

We have previously shown that Ox-LDL, particularly at low concentrations, can exert a concentration-dependent stimulation in the proliferation of aortic smooth muscle cells, (Chatterjee, 1992). Subsequent studies from our laboratory revealed that lactosylceramide alone could stimulate the proliferation of these aortic smooth muscle cells (Chatterjee, 1991). These findings are of great biological and clinical significance as Ox-LDL has been implicated in the pathogenesis of atherosclerosis. Moreover, recent studies from our laboratory reveal that both Ox-LDL and lactosylceramide are enriched in the atherosclerotic plaque in human subjects who have died of coronary artery disease (Chatterjee, S., Dey, S., Shi, W.Y., Thomas, K. and Hutchins, G., unpublished observations). Accordingly, we sought to understand biochemical mechanisms involved in the Ox-LDL mediated proliferation of aortic smooth muscle cells. Our studies reported in this manuscript reveal that indeed Ox-LDL at low concentrations can stimulate the proliferation of aortic smooth muscle cells. This phenomenon (Figure 7) may involve the binding of Ox-LDL to a putative cell surface receptor. Such a receptor has been recently described by Steinberg and co-workers (unpublished observations). This tenet is also supported with the evidence that the binding of Ox-LDL at 4°C does not stimulate the activity of GalT-2. Moreover, Ox-LDL did not impart any effect on the activity of GalT-2 in cell-free extracts. Taken together, these findings imply that Ox-LDL may be taken

\[ \begin{array}{|c|c|c|} 
\hline
\text{Treatment} & \text{GalT-2 activity} & \% \text{Control} \\
& \text{nmol/mg protein/2 hr} & \text{value} \\
\hline
\text{Control} & 2.75 \pm 0.15 & 100 \\
\text{Ox-LDL} & 3.85 \pm 0.15 & 140 \\
\text{Anti-Ox-LDL + Ox-LDL} & 2.85 \pm 0.25 & 103 \\
\text{Anti-Ox-LDL} & 2.75 \pm 0.25 & 100 \\
\text{Anti-LacCer + Ox-LDL} & 3.80 \pm 0.20 & 138 \\
\text{Anti-GalT-2} & 2.70 \pm 0.20 & 98 \\
\text{Anti-GalT-2 + Ox-LDL} & 2.90 \pm 0.20 & 105 \\
\hline
\end{array} \]

Cells were incubated for 1 hr with various antibodies (dilution 1:100). Next, Ox-LDL (10 \( \mu \text{g/ml} \)) was added. After incubation for 2 hr, cells were harvested and the activity of GalT-2 was measured. The data represent standard deviation values from two separate experiments analyzed in duplicate.

Table 1. Effects of various antibodies on the activity of GaIT-2 in cultured aortic smooth muscle cells
cDNA probes for GalT-2 will help us understand the regulatory elements involved in Ox-LDL mediated activation of this enzyme and LDL mediated suppression of this enzyme in a variety of cultured cells. Such studies will hopefully lead to a better understanding of biochemical mechanisms involved in Ox-LDL mediated proliferation of aortic smooth muscle cells, a hallmark in the pathogenesis of atherosclerosis.

Materials and methods

**Materials**

Methyl-[3H]-thymidine (specific activity 92.5 GBq/mmol), uridine diphosphate galactose (specific activity 12.2 GBq/mmol), d-[3H]-galactose (specific activity 1.3 TBq/mmol), and l-[3H]-serine (specific activity 1.07 TBq/mmol), were purchased from Amersham, Inc. D-PDMP and L-PDMP were from Matreya, Inc. All other chemicals were purchased from Sigma Chemical Co.

Aortic smooth muscle cells from New Zealand White rabbits were prepared as described previously (Ross, 1971). Large batches of primary cultures were frozen and all experiments were done within passage 2–3. Typically, cells (x10^6) were seeded in P-100 plastic petri dishes and grown in minimum essential medium containing 10% fetal calf serum, nonessential amino acids, glutamine, penicillin, and streptomycin. Medium was changed on the 3rd and 6th day of cell growth. On the 7th day of cell growth, medium was removed and cells were incubated with serum-free Ham's-F-10 medium for 2 h. Then various compounds were added, incubated, and processed as described below. For cell proliferation assay, cells (x10^5) were seeded in 96-well microtiter plates.

**Preparation of LDL, oxidized LDL, and ^125I-Ox-LDL**

Human plasma low density lipoproteins were prepared employing KBr gradient centrifugation (1.019–1.063 g/ml) as described previously (Havel, et al., 1955). Ox-LDL was prepared by dialyzing LDL against 5 mM CuSO4 in sterile phosphate-buffered saline. LDL and Ox-LDL were characterized using physical-chemical criteria including electrophoretic migration on agarose gels, assays for thiobarbituric acid positive substance and lysophosphatidylcholine, phosphatidyleholine levels as described (Chatterjee, 1992). ^125I-LDL was prepared using modification of the procedure of McFarlane as described by us previously (Chatterjee, et al., 1988). ^125I-Ox-LDL was prepared from LDL by dialyzing against a solution of 5 μM copper sulfate in phosphate buffered saline. Freshly prepared Ox-LDL was used immediately in our experiments.

**Incubation of cells with Ox-LDL and LDL**

Following incubation of cells with Ham's F-10 medium, freshly prepared Ox-LDL and LDL (0–100 μg protein/ml) was added and incubation carried out for 1 h. Cells were washed with PBS, harvested and stored frozen. In another set of dishes, cells were incubated with Ox-LDL (10 μg protein/ml) for 0, 5 min, 10 min, 15 min, 30 min, 45 min, 60 min, 2 h, and 4 h. Next, cells were harvested in cacodylate buffer (pH 6.8) centrifuged and the pellets stored at −20°C. Such cell pellets were used for the measurement of various enzyme activities.

**Preparation of antibodies**

Polyclonal antibodies against human kidney UDP-galactose: glucosylceramide, β1 → 4 galactosyltransferase (GalT-2), lactosylceramide and human plasma oxidized LDL were prepared against New Zealand White rabbits as described previously (Chatterjee, 1989). Immunoglobulin G were prepared from the respective preimmune serum using ion exchange column chromatography (Econo-pac serum IgG purification kit, Bio-Rad, Richmond, CA). Next, the antibodies were immunosorbed against antigen and the monospecific polyclonal antibodies were eluted as described previously (Chatterjee and Ghosh, 1989).

**Assay for GalT-2, other glycosyltransferases, and glycohydrolases**

Cells incubated with and without Ox-LDL, LDL, and other compounds were homogenized and suitable aliquots were used to measure the following activities: UDP-glucose:ceramide β1 → 4 glycosyltransferase (GlcT-1), GalT-2, GbOse,Cer 1 → 4 galactosidase, and LacCer β1 → 4 galactosidase. The procedures used for such assays have been described previously (Chatterjee, et al., 1988).
Incubation of cells with D-PDMP and L-PDMP

Stock solutions (100 μM) of D-PDMP and L-PDMP were prepared in isopropanol. Suitable aliquots (10 μl/ml) of these solutions were added to cells preincubated with Ham’s F-10 medium. After incubation for 1 h, 2 h, 4 h, and 24 h, cells were harvested and the activity of GalT-2 measured.

Incubation of cells with various antibodies and Ox-LDL

Confluent monolayers of cells were incubated with Ham’s F-10 medium for 2 h at 37°C. Next, GalT-2 antibody, LacCer antibody, Ox-LDL antibodies (10 μl each) were added to cells. After 1 h at 37°C Ox-LDL (10 μg/ml) was then added to each dish. The cells were incubated further for 2 h at 37°C, harvested and subjected to GalT-2 measurements.

Measurement of cell proliferation

Cells were grown in 96-well microtiter plates for 6 d. On the sixth day medium was removed with fresh Ham’s F-10 ml (100 μl) with and without Ox-LDL, and antibodies. After incubation for 22 h at 37°C, [3H]-thymidine (5 μCi/ml) was added and incubation continued for an additional 2 h. Next, cells were washed with PBS and the incorporation of [3H]-thymidine was measured as described (Chatterjee et al., 1992).

Incorporation of [3H]-galactose and [3H]-serine into glycosphingolipids

Cells (×10⁴) grown in 6-well plastic trays for 6 days were given Ham’s F-10 medium with and without Ox-LDL (0-200 μg/ml) and [3H]-Galactose or [3H]-serine (5 μCi/ml). After incubation for 24 h, cells were harvested in PBS and centrifuged. The incorporation of radioactivity into individual glycosphingolipids was pursued following extraction of total lipids, silicic acid column chromatography, HPTLC separation, and scintillation spectrometry as described previously (Chatterjee et al., 1986).

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Abbreviations

Ox-LDL, oxidized low density lipoproteins; LDL, low density lipoproteins; GlcCer, glucosylceramide; LacCer, lactosylceramide; D-PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PBS, phosphate-buffered saline; GalT-2, UDP-galactose; GlcCer β1-4 galactosyltransferase.

References


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