Histamine increases the expression of LOX-1 via H2 receptor in human monocytic THP-1 cells

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Abstract  Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is a member of the scavenger receptor family, and is known to be expressed in the monocytes/macrophages. We investigated the effect of histamine on the expression of LOX-1 in cells of the human monocytic leukemia cell line THP-1. Histamine as well as forskolin and dibutyryl cyclic AMP (Bt\textsubscript{2}-cAMP) stimulated the THP-1 monocytes to express the LOX-1 gene at the transcription level. This histamine effect on LOX-1 gene expression, via the histamine H2 receptor-mediated cAMP signal transduction pathway, was reduced after differentiation of the cells into macrophages, even though forskolin and Bt\textsubscript{2}-cAMP still enhanced the gene expression. The alteration of the responsiveness of LOX-1 expression to histamine was related to suppressed expression of the H2 receptor in THP-1 macrophages. The switch of the predominant class of histamine receptors between H1 and H2 would modulate the effects of histamine on LOX-1 gene expression in monocytes and macrophages, and therefore, would play a certain role in the inflammatory aspects of atherogenesis.

Key words: Monocyte/macrophage; Histamine receptor; Lectin-like oxidized low-density lipoprotein receptor-1; Cyclic AMP response element; Atherosclerosis

1. Introduction

Histamine is synthesized from L-histidine by histidine decarboxylase (HDC) in a single enzymatic step, and it is one of the principal mediators related to allergic reactions, inflammation [1–3], neurotransmission [4] and cardiovascular hemodynamics [5]. Many types of cells express specific histamine receptors, which are classified into H1, H2, and H3 types [6–8]. The H1 receptor couples to G\textsubscript{q} and the phosphoinositol hydrolysis pathway, while the H2 receptor links to G\textsubscript{s} to activate adenylate cyclase. Recently, a gene for the histamine H3 receptor, the product of which couples to G\textsubscript{i}, has been cloned [9], and the presence of another type of histamine receptor, H4 receptor, has also been reported [10,11]. We previously reported that histamine H1 receptor is predominantly expressed in vascular smooth muscle and endothelial cells [11] and that histamine stimulates vascular smooth muscle cells to proliferate via the H1 receptor [12]. Furthermore, our recent work demonstrated that a switch of histamine receptor from H2 to H1 takes place during macrophage differentiation and that this receptor switch is closely related to regulation of lipopolysaccharide-induced expression of tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) by histamine [13]. Knowledge about the diversity of histamine receptors expressed on the cells constituting the atherosclerotic vascular wall would be very important for a better understanding of the physiological and pathological roles of histamine.

Monocytes/macrophages play a central role in atherogenesis, and they exhibit multiple functions in relation to the formation of the atherosclerotic lesion, which include monocytic migration and secretion of growth factors, cytokines, and matrix-degrading enzymes [14]. On the other hand, scavenger receptors are key molecules that allow monocytes/macrophages to take up modified lipoproteins and to become transformed into foam cells [15]. Many types of scavenger receptors are reported to exist on the monocyte/macrophage [15], and the lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is the major receptor for oxidized low-density lipoprotein (LDL) [16,17]. Inflammatory and immunologic events are involved in the pathogenesis of atherosclerosis, and oxidized LDL is one of the activators of T-lymphocytes and macrophages in the vascular wall [18–20]. In fact, expression of LOX-1 has been reported in endothelial cells, macrophages, and smooth muscle cells in the intima of the atherosclerotic lesion [21]. We recently demonstrated that activated monocytes and T-lymphocytes expressed HDC mRNA in vitro and in atheromatous plaques as a non-mast cell source of histamine [22]. Therefore, it is necessary to examine the effects of histamine on LOX-1 gene expression to understand the mechanism of initiation and progression of atherosclerosis.

In the present study, we demonstrated that LOX-1 gene expression is regulated through the cyclic AMP signaling pathway and that histamine up-regulates the LOX-1 expression via the H2 receptor in THP-1 monocytes.

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2. Materials and methods

2.1. Chemicals

Forskolin, protein kinase A (PKA) inhibitor (HS9), nitroblue tetrazolium, and tetradeoxyadenosine-3'-13-acetate (TPA) were obtained from Sigma (St. Louis, MO, USA). Histamine was from Wako Pure Chemical Industries (Osaka, Japan). Dibutyryl cyclic AMP (Bt2cAMP) was from Biomol Research Laboratories (Plymouth Meeting, PA, USA); and the histamine H2 recepter agonist dimaprit dihydrochloride was from Tocris Cookson Ltd. (Ballwin, MO, USA). Anti-human CD68 mouse monoclonal antibody for flow cytometric analyses was purchased from BD PharMingen (San Diego, CA, USA).

2.2. Cell culture

The human monocyte leukemic cell line THP-1 was obtained from American Type Culture Collection (TIB-202) and maintained in RPMI 1640 medium (Sigma) containing 5% fetal calf serum (FCS, ICN, Costa Mesa, CA, USA) at 37°C in an atmosphere of 95% air and 5% CO2.

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNAs extracted from the THP-1 cells were subjected to reverse transcriptase reaction and PCR, and the primers for amplification of histamine H1 and H2 receptor cDNAs were designed and used for PCR as previously reported [13]. For PCR amplification of histamine H3 (GenBank accession number AL078633) and H4 (GenBank accession number NM021624), two sets of primers, 5'-TCTTCTGCTCTCAAATCAGC-3' and 5'-ATCATCAGCAGCGTGT-3' (to generate a 779-bp fragment) and 5'-GAATTGTCTGGCCTAGTTAAGAATGATGGAAGGTTAGTGGAAGAAGGTTACAC-3' (to generate a 1003-bp fragment), were used, respectively. Denaturation, annealing and extension temperatures were 35 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min, for H3 receptor, and 2 times repeated PCRs of each 20 cycles at the same temperature condition of H3, for H4 receptor. All the amplified fragments were cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced.

2.4. Northern blotting

The THP-1 cells were stimulated with TPA (20 ng/ml) for 24 h in RPMI 1640 containing 5% FCS and further incubated in RPMI 1640 containing 0.5% FCS for 48 h for differentiation into macrophages. Then the THP-1 macrophages were stimulated with histamine (50 μM), forskolin (10 μM) or Bt2-cAMP (200 μM) for the desired periods. Monocytic THP-1 cells were also used for the same experiment. Total RNAs were extracted by Trizol reagent (Gibco BRL, Rockville, MD, USA) and electrophoresed on 1% agarose gel containing 6% agarose gel (for histamine H3 and H4) or 8% agarose gel (for histamine H1) for 2 h at 80V. The membranes were exposed to X-ray film at -80°C overnight or longer. Equal loading of the RNAs for each sample was confirmed by methylene blue (0.02% in 0.5 M sodium acetate, pH 5.2) staining of the membrane to visualize 28S and 18S ribosomal RNAs (data not shown). The amplification histamine receptor cDNA fragments (H1, H2 and H4) were also used for probes of Northern blotting after 3P labeling.

2.5. Cloning of LOX-1 promoter

Based on the reported sequence data (GenBank accession number AB021922) of the human LOX-1 promoter region [22], a 1053-bp fragment corresponding to nucleotides -1017 to +36 was generated by PCR from genomic DNA of the THP-1 cells as a template. After the sequence had been confirmed, the fragment was ligated to a luciferase reporter vector, pGL3-basic (Promega) at Mfd and HidIII sites (pLOX-Luc). A pLOX-CREM-Luc construct was generated by a substitution of GST to CA (112 and -11) by site-directed mutagenesis to introduce a mutation in the cyclic AMP response element (CRE).

2.6. Transfection and luciferase assay

The monocytic THP-1 cells (1.4×10^4/0.5 ml/cuvette) were transfected with 20 μg of reporter constructs along with 0.5 μg of β-gal expression vector by electroporation at a setting of 950 μF and 300 kV (GenePulsar II, Bio-Rad, Hercules, CA, USA). The cells were lysed and mixed with luciferase substrate (Toyo Ink, Tokyo, Japan) for measurement of luciferase activity 8 h after the electroporation. To decrease variation in transfection efficiency, we transfected the cells in a single batch for each reporter plasmid, which was then separated to the desired numbers of wells. Further, β-gal activity was measured to monitor the transfection efficiency. For co-transfection studies, pMT-Pkα (gift from Dr. Marc Montminy [24]) and pCREB341 (gift from Dr. Richard H. Goodman [25]) were used for the luciferase reporter assays at a dose of 1.0 and 2.0 μg per electroporation cuvette, respectively.

2.7. Analyses for macrophage differentiation

To investigate macrophage differentiation by histamine, we examined the expression of CD68 and capacity for NBT reduction. The monocytic THP-1 cells were incubated with histamine (50 μM) for 3 days and then subjected to flow cytometry (Epic XL, Beckman Coulter, Miami, FL, USA) and NBT reduction assay. As a positive control for macrophage differentiation, the THP-1 cells were stimulated with Bt2-cAMP (200 μM) for the same period.

3. Results and discussion

Many genes are expressed in accordance with macrophage differentiation [26], and LOX-1 expression has been reported to increase during TPA-induced macrophage differentiation of THP-1 cells [27]. Our results confirmed that the THP-1 cells after differentiation into macrophages expressed LOX-1 mRNA (Fig. 1A, row 3) and showed that the basal expression in the monocytic condition was very low (Fig. 1A, row 1). However, longer exposure of the Northern blot membrane revealed that the monocytic THP-1 cells also expressed LOX-1 mRNA and that the expression was induced by histamine (Fig. 1A, row 2). No mRNA induction by histamine over the control level was detected in the THP-1 macrophages (Fig. 1A, row 3). In contrast, cAMP-elevating agents, forskolin and Bt2-cAMP, apparently enhanced the expression of LOX-1 mRNA in both monocytic and macrophage THP-1 cells. This histamine-induced LOX-1 mRNA expression in monocytic THP-1 cells was observed as early as 2 h after the start of stimulation (Fig. 1B). These results indicate that...
LOX-1 expression is up-regulated by cAMP-elevating agents, including histamine, the H2 receptor of which couples to Gs to elevate the cytoplasmic concentration of cAMP.

To determine whether the differences in histamine responsiveness between the monocytes and macrophages resulted from expression of different receptor types, we applied the mRNAs to RT-PCR to obtain the receptor expression profile. As shown in Fig. 2A, the monocytic THP-1 cells expressed a higher amount of H2 receptor than of H1 receptor. In contrast, after macrophage differentiation by TPA treatment, the H1 receptor expression was induced and the H2 receptor expression was reduced. Histamine H3 receptor mRNAs were not detected in either condition, whereas H4 receptor expression was very faintly detected in macrophages (arrowhead, Fig. 2A). To confirm the receptor expression profile in the THP-1 cells, we performed Northern blot analysis using H1, H2, and H4 receptor cDNA probes. The switch of the predominant histamine receptor type from H2 to H1 during macrophage differentiation was clearly demonstrated by this analysis (Fig. 2B). H4 receptor expression was not detected in our experimental conditions (data not shown). Fig. 3A shows the quantitative analysis of the receptor expression profile in response to differentiation. The histamine receptor profile was H2 dominant in the monocytic condition, while it was H1 dominant in the macrophage condition (Fig. 3A). This pattern is exactly the same as that observed in another human monocytic cell line, U937, in which histamine receptor switching is exactly the same as that observed in another human monocytic cell line, U937, during macrophage differentiation [13]. The responsiveness of LOX-1 mRNA expression to histamine was higher in the monocytic condition (Fig. 1). The responsiveness of LOX-1 mRNA expression to histamine is higher in monocytic THP-1 cells than in macrophages (Mφ).

Fig. 2. RT-PCR and Northern blot analyses of histamine receptor gene expression in THP-1 cells. H1, H2, H3, and H4 refer to histamine H1, H2, H3, and H4 receptor, respectively. A: In the monocytic condition (TPA−), H2 receptor expression is dominant; in contrast, H2 expression is reduced in the macrophage condition (TPA+). No H3 receptor expression was detected, whereas a low level of H4 receptor mRNA expression was noted (arrowhead). Amplified fragments of H1, H2, and H4 receptor cDNA were 492, 498, and 596 bp, respectively. M, 100-bp ladder DNA size marker. B: Northern blot analysis confirmed the predominant H2 receptor expression in the monocytes (TPA−); in contrast, the H1 receptor was predominant in the macrophages (TPA+).

Fig. 3. Quantitative analyses of histamine receptor profile and histamine responsiveness. A: Ratio of H2 and H1 receptor expression was calculated by densitometric analysis of the Northern blot (Fig. 2B). Again note that the histamine receptor expression profile is predominantly of the H2 type in the monocytic condition. B: Fold activation by histamine stimulation over the control level of LOX-1 mRNA expression (Fig. 1) was calculated following densitometry of the histamine responsiveness. Note that the response of LOX-1 mRNA expression to histamine is higher in monocytic THP-1 cells than in macrophages (Mφ).

To address whether histamine transcriptionally up-regulates the LOX-1 gene in the monocytic THP-1 cells through the cAMP signal transduction pathway, we constructed a luciferase reporter plasmid including the human LOX-1 promoter region (pLOX-Luc, Fig. 4A). The cloned promoter region, spanning from −1017 to +36 of the transcription initiation site, includes some enhancer elements, but a proximal fragment of 180 bp (−150 to +30) has been reported to be sufficient to regulate basal promoter activity [23]. This 180-bp fragment contains an inverted CRE at −116 to −109, which overlaps a GC box. As shown in Fig. 4B, all the cAMP-elevating agents including histamine and H2 receptor agonist increased the transcriptional activity from the pLOX-Luc (wild type). Histamine H1 and H3 agonists had no effect on the activity (data not shown). When THP-1 cells were transfected with pLOX-CREM-Luc (CREm) harboring a mutation in the CRE (GT to CA), the basal activity was reduced to 20% of that of pLOX-Luc and no induction by the cAMP-elevating agents was observed (Fig. 4B), indicating that the CRE motif was essential for both basal activity and the response to cAMP-elevating agents. It was reported earlier that LOX-1 mRNA was up-regulated by TNF-α and transforming growth factor-β1 in mouse macrophages [17,27], but the molecular mechanism by which the expression of the LOX-1 gene is regulated yet to be clarified.

Next we investigated the effect of H89, a PKA inhibitor, on the promoter activity to confirm the involvement of the cAMP-PKA-CRE pathway for LOX-1 gene regulation. The H89 treatment reduced the histamine- and H2 agonist-induced promoter activities and basal activity in a dose-dependent manner (Fig. 4C). Along with the CRE mutational analysis, these results suggest the involvement of PKA and its substrate, CRE binding protein (CREB), for the basal and inducible activity of the LOX-1 gene promoter. To clarify this further, we introduced PKA and CREB expression vectors into the luciferase reporter assays. The MT-I promoter drives the PKA expression vector, pMT-Pka [24], and the expression is controlled by the addition of zinc ions to the culture medium.
The CREB expression vector, pCREB341, is driven by Rous sarcoma virus promoter [25]. Either PKA or CREB over-expression up-regulated the LOX-1 gene transcription, and co-transfection further enhanced the promoter activity (Fig. 5, Wild type). This PKA and CREB effect was not detected when the cells were co-transfected with pLOX-CREM-Luc as a reporter (Fig. 5, CREm).

Here we demonstrated that histamine up-regulated LOX-1 gene expression via the H2 receptor in THP-1 cells in the monocytic state. Since LOX-1 might be a differentiation-related gene preferentially expressed in macrophages [17,27] and a report indicated H2 receptor-mediated cellular differentiation of the myelogenic leukemia cell line HL60 [28], we studied the possibility that histamine might induce macrophage differentiation of THP-1 cells. However, even with a 3-day incubation with 50 μM histamine, the monocytic THP-1 cells did not differentiate into macrophages when the cells were monitored by CD68 expression, NBT reduction, and morphological changes as differentiation markers (data not shown). Similarly the H2 agonist dimaprit had no effect on cellular differentiation in U937 monocytic cells [29].

Since both histamine and prostaglandins are secreted from monocytes/macrophages and are present in the environment of atherosclerotic lesions [22,30–32], we also investigated the effect of PGE2 and PGD2 on LOX-1 gene expression. Northern blot analysis revealed that PGE2 stimulated the macrophages, but not the monocytic THP-1 cells, to enhance LOX-1 mRNA expression (Fig. 6). This contrasts with the response to histamine found in monocytic THP-1 cells (Fig. 1). These inflammatory mediators, histamine and PGE2, would thus play a role in modulating LOX-1 gene expression in monocytes and macrophages, respectively. Interestingly, both histamine and PGE2 inhibited production of the Th1-inducing cytokine interleukin (IL)-12 from monocytes [33,34]; and, in
addition, they inhibited IL-2 and interferon-γ (IFN-γ) (Th1 cytokines) but not IL-4 and IL-5 (Th2 cytokines) production from T-lymphocytes [35–37]. These findings indicate that histamine suppresses the Th1 immune response in atherosclerotic lesions, whereas the Th1-type response is predominant [38,39]. On the other hand, it was reported that Th2 cytokines (IL-4 and IL-13) stimulated monocytes to oxidize LDL and that, in contrast, IFN-γ inhibited the oxidation [40]. Taken together with our results, these findings suggest that histamine may promote monocyte-oxidation of LDL through Th1 suppression and enhance uptake of oxidized LDL via increased expression of LOX-1. This seems contrary to the fact that IFN-γ suppressed the Th1 immune response in atherosclerotic lesions but not IL-4 and IL-5 (Th2 cytokines) production [41]. However, the net effects of histamine on the immune response in the atherosclerotic lesion are still controversial, because oxidized LDL itself stimulates monocytes to produce IL-12 [38]. Our observation suggests a potential contributing role of histamine to LOX-1 expression in relation to immunology, inflammation, and atherosclerosis.

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References