Sphingosine kinase inhibition exerts both pro- and anti-atherogenic effects in low-density lipoprotein receptor-deficient (LDL-R−/−) mice

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Summary

Sphingosine 1-phosphate (S1P), a lysosphingolipid associated with high-density lipoprotein (HDL), contributes to the anti-atherogenic potential attributed to this lipoprotein. This study examined whether a reduction of S1P plasma levels affects atherosclerosis in a murine model of disease. LDL-R−/− mice on Western diet were given ABC294640, an inhibitor of sphingosine kinase (SphK) for 16 weeks. ABC294640 decreased plasma S1P by approximately 30%. However, ABC294640 failed to affect atherosclerotic lesion formation. Plasma triglycerides were reduced whereas total and HDL-cholesterol remained unchanged in course of ABC294640 treatment. ABC294640 increased plasma interleukin (IL)-12p70 and RANTES concentration as well as IL-12p70, RANTES and interferon (IFN)-γ production by peritoneal cells and this was paralleled by enhanced activity of peritoneal and spleen dendritic cells as evidenced by up-regulation of CD86 and MHC-II on CD11c+ cells. As a consequence, increased T-cell activation was noted in ABC294640-treated mice as indicated by enhanced CD4+ splenocyte proliferation, IFN-γ and IL-2 production, and CD69 expression. Concomitantly, however, ABC294640 treatment redistributed CD4+ and CD8+ cells from blood to lymphatic organs and reduced T-cell number within atherosclerotic lesions. In addition, plasma sVCAM-1, sICAM-1, and MCP-1 levels as well as in vivo leukocyte adhesion and CCL19-induced T-cell penetration into peritoneum were lower in ABC294640-treated animals. In vitro experiments demonstrated reduced VCAM-1 and ICAM-1 expression and lymphocyte adhesion to endothelial cells exposed to ABC294640. In conclusion, treatment with SphK inhibitor leads to both pro- and anti-atherogenic effects in LDL-R−/− mice. As a consequence, SphK inhibition fails to affect atherosclerosis despite significant S1P reduction in plasma.

Keywords

Sphingosine 1-phosphate, high density lipoproteins, inflammation, endothelium, animal models of atherosclerosis

Introduction

Sphingosine 1-phosphate (S1P) is an important lipid mediator generated from phospholipids upon cell activation and is present in plasma and extracellular fluid in high nanomolar concentrations (200–1,000 nM) (1, 2). S1P is produced by phosphorylation of sphingosine by sphingosine kinases (SphK) 1 and 2 in response to a variety of stimuli and is actively exported out of the cells (inside-out signalling), where it serves as a ligand for five G protein-coupled receptors termed S1PR1–5 (3–5). Extracellular S1P exerts modulatory effects on diverse processes in the vasculature including survival, proliferation, migration and cell-cell adhesion of endothelial and smooth muscle cells and thereby controls endothelial barrier integrity, vascular development and vessel contraction (6, 7). Moreover, S1P regulates lymphocytes and monocytes trafficking between lymphoid compartments and peripheral sites of inflammation and promotes development of anti-inflammatory functional cell phenotypes (Th2, M2) (8–10). In addition to extracellular effects S1P assumes a role as an intracellular second messenger and promotes expression of cytokines and adhesion-mediating molecules such as VCAM-1 and ICAM-1 in macrophages and endothelial cells, respectively (1, 11, 12). Due to these pleiotropic effects exerted both extra- and intracellularly, S1P is critical for the maintenance of vascular homeostasis, while its perturbations may be decisive for the emergence of inflammatory vascular diseases such as atherosclerosis.
Recent investigations provide circumstantial evidence suggesting that S1P may be directly involved in the pathogenesis of atherosclerosis. For instance, extracellular S1P is mainly contained in high-density lipoproteins (HDL) – a potent plasma-borne anti-atherogenic factor, and decreased HDL-bound S1P levels have been observed in patients with coronary artery disease and myocardial infarction (13, 14). In addition, S1P was found to emulate in vitro several atheroprotective effects attributed to HDL, including inhibition of endothelial apoptosis and stimulation of endothelial cell movement, inhibition of the expression of adhesion molecules and stimulation of nitric oxide (NO) and prostacyclin generation (15–18). FTY720, a synthetic S1P analogue and a high affinity agonist for S1PR1, 3, 4, and 5 was demonstrated to retard atherosclerotic lesion formation in both low-density lipoprotein (LDL) receptor-deficient (LDL-R−/−) and apolipoprotein E-deficient (apoE−/−) mice fed cholesterol-rich Western diet (19, 20). However, the contribution of endogenous S1P to the development of atherosclerosis has not been investigated to date. To address this issue, we studied the development of atherosclerosis in LDL-R−/− mice exposed to ABC294640, a potent and specific SphK inhibitor (21, 22). Our results demonstrate that the SphK inhibition and the ensuing reduction of plasma S1P levels lead to both pro-atherogenic effects (via enhanced activation of dendritic cells and T-cells) and anti-atherogenic effects (via reduced activation of endothelial cells) that mutually equalise. As a consequence, SphK inhibition does not affect atherosclerotic lesion formation in LDL-R−/− mice.

### Intravital microscopy

For studying leukocyte adhesion under in vivo conditions, LDL-R−/− mice were put on Western diet and administered ABC294640 (75.0 mg/kg/day) or saline for seven days. Leukocyte adhesion was stimulated by i.p. injection of 5.0 mg/kg endotoxin. After 6 hours (h), leukocytes were stained perivitally with 1.0 mg/kg of carboxyfluorescein diacetate succinimidyl ester (CFDA-SE, Molecular Probes, Leiden, Netherlands) by intravenous injection of the fluorochrome solution as described (23). Animals were anesthetised with 2% isoﬂurane, and leukocyte adhesion in mesenterial venules was assessed by intravital microscopy of small mesenterial venules (diameter 20–30 μm). For this purpose, a small midline laparotomy was performed in the lower abdomen, animals were placed on their right side on an inverted fluorescence microscope (Eclipse 300, Nikon, Düsseldorf, Germany), and a part of the distal ileum was gently exteriorised. Mesenteric venules with a diameter of 20–30 μm were visualised and recorded for 60 seconds (sec) by a fluorescence camera (FView II Olympus Soft Imaging Systems, Münster, Germany) on a computer for offline evaluation. Temporary leukocyte adhesion was defined as movement of a leukocyte with markedly reduced speed or adhesion < 20 sec to the endothelium. Permanent adhesion was defined as leukocytes without movement > 20 sec. The numbers of leukocytes were normalised on 100 μm length of venular wall.

### Histology and lesion analysis

Exsanguinated animals were subjected to in situ perfusion with saline through the left cardiac ventricle. For analysis of spontaneous atherosclerosis aortic roots were removed and embedded in Tissue-Tek. Transverse 10 μm cryosections were prepared, mounted in order on series of slides, and stained with Oil Red O and hematoxylin (Sigma). Cross-sections with maximal stenosis were used for morphometric analysis on a DM-RE microscope with Leica Qwin image analysis software (Leica Microsystems B.V., Rijswijk, the Netherlands), as described previously (19). Corresponding sections were stained immunohistochemically with antibodies directed against mouse macrophages (monoclonal mouse IgG2a, clone MOMA-2, dilution 1:50; Sigma Diagnostics, St. Louis, MO, USA) and lymphocytes (purified anti-mouse CD3 clone SP7, dilution 1:50; Immunologic, Duiven, The Netherlands). Macrophages positive areas were determined by computer-assisted colour-gated measurement, and related to the total intimal surface area. For lymphocytes, the number of CD3-positive cells was assessed within plaque areas in five consecutive sections, and averages were used for analysis.

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**Lipid analysis**

Plasma total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) were determined enzymatically using commercially available kits (Roche, Mannheim, Germany). S1P levels were determined after extraction and derivatisation with o-phthalaldehyde using a Merck-Hitachi LiChrom HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with RP18 Kromasil column (Chromatographie Service GmbH, Langerwehe, Germany) as previously described (24). The S1P recovery was calculated using dihydro-S1P as a standard.

**Differential cell immunophenotyping**

Peritoneal leukocytes and single-cell suspensions of spleen and lymph nodes were immunophenotyped by flow cytometry (FACScalibur, BD Bioscience, San Jose, CA, USA). Monoclonal antibodies for flow cytometry were from BD Bioscience, Heidelberg, Germany (CD4, CD8 and CD69) or ebioscience, Frankfurt, Germany (CD8, CD19, CD62L, CD11b, CD11c, F4/80, MHCII and CD86). For each FACS staining 2x10⁵ cells were incubated with antibody dilutions (0.25 μg for each antibody) in PBS plus 1.0% (v/v) FCS at 4°C.

**Tissue harvesting**

For chemotaxis studies mice received i.p. injections of CCL19 (500 ng/ml; Peprotech, Rocky Hill, NJ, USA) 16 h before sacrifice. Control mice were injected with PBS. At sacrifice mice were anaesthetised and bled by orbital exsanguination. Peritoneal leukocytes were isolated by peritoneal lavage (ice-cold PBS) as described previously (19). Cells were suspended in DMEM containing FCS (10.0% v/v) and 2 mM glutamine and were either used for flow cytometry or seeded in a 24-well plate at a density of 0.5 x 10⁶ cells/well. After 4 h non-adherent cells were removed and remaining macrophages were incubated for 24 h in the absence or presence of lipopolysaccharide (LPS; 50 ng/ml; Sigma). Spleens and lymph nodes (skin and mesenteric) were excised and single-cell suspensions were prepared by passing crude cell suspensions through a 70 μm mesh filter. Erythrocytes in cell suspensions were lysed by hypo-osmotic shock as described above.

Murine endothelial cell line bEnd.5 was a generous gift of Dr. Sigrid März (Max-Planck-Institute for Molecular Medicine, Münster, Germany) and was maintained in DMEM supplemented with glutamine (2.0%, v/v), sodium pyruvate (1.0%, v/v), non-essential amino acids (1.0%, v/v), FCS (20.0%, v/v), and endothelial cell growth supplement (PromoCell, Heidelberg, Germany) containing human basic fibroblast growth factor, epithelial growth factor and porcine heparin. Murine lymphoma cell line EL4 was obtained from Sigma and was maintained in DMEM with FCS.
For the assessment of lymphocyte adhesion, EL4 cells were labelled for 30 min at 37°C with fluorescent dye calcein (1.0 μM, Molecular Probes) and added at a number of 4 x 10^6 cells/ml to a confluent bEnd.5 monolayer for 30 min under gentle rocking. Non-adherent cells were removed thereafter by rinsing plates three times and the number of adherent cells was counted under fluorescence microscope Leica DM-IRE (Leica Mikrosysteme, Wetzlar, Germany) with at least five fields per cover slip being quantified.

Western blotting

Endothelial cells were lysed in a buffer containing 50 mM Tris-HCl, 0.15 M NaCl, 1.0% (v/v) Nonidet P-40, 5.0% (v/v) sodium deoxycholate, 0.1% (v/v) SDS, 300 mM NaF, 1.0 mM EGTA, 1.0 mM orthovanadate, and protease inhibitors (Complete, Roche). Cell lysates (50 μg/lane) were subjected to SDS-polyacrylamide gel electrophoresis. Thereafter proteins were transferred to nitrocellulose membranes, which were blocked overnight in Tris-buffered saline containing 5.0% non-fat dry milk prior to incubations with antibodies against VCAM-1 and ICAM-1 (both AB-Biotech, San Diego, CA, USA).

Statistical analysis

Data are presented as means ± standard deviation (SD) for at least three separate experiments or as results representative for at least three repetitions. Comparisons between the means of two groups were performed with two-tailed Student t-test. Comparisons between the means of multiple groups were made by one-way ANOVA for independent samples. Pairwise comparisons were performed thereafter with Student-Newman-Keuls post-hoc test. P-values less than 0.05 were considered significant.
Results

SphK inhibition reduces plasma triglycerides and S1P levels

ABC294640 is a synthetic SphK inhibitor with IC50 of 60.0 μM for SphK2 and above 100 μM for SphK1, and that can be chronically administered to mice without systemic toxicity (21). Here, ABC294640 was administered five-weekly via i.p. injections to eight-week-old LDL-R–/– mice for a period of 16 weeks at a dose previously demonstrated to effectively suppress dextran sulfate-induced colitis and diabetic retinopathy (22, 26). Body weight did not differ between groups when fed a cholesterol-enriched Western type diet (not shown). Similarly, both total and HDL cholesterol remained unaltered (Fig. 1). However, there was a considerable decrease in triglyceride levels in the ABC294640-treated group. As expected, administration of ABC294640 significantly reduced plasma S1P levels by approximately 30%.

SphK inhibition fails to affect plaque size but alters cellular composition

Lesion size was measured to determine the effect of SphK inhibition on the diet-induced atherogenesis. Morphometric analysis of the Oil Red O-stained aortic root did not show any effect of ABC294640 on the absolute area of the plaque (Fig. 2A) or on the plaque-to-lumen ratio (not shown). Immunohistochemical analysis of lesion composition yielded no significant differences with regard to MOMA-positive macrophage content (Fig. 2B). Further analysis, however, showed that treatment with ABC294640 substantially reduced plaque content of CD3-positive T cells – an important determinant of the intraplaque inflammation (Fig. 2C).

SphK inhibition affects lymphocyte profile and enhances T cell activation

Numerous studies revealed regulatory influence of S1P on lymphocyte distribution and T cell function (8, 9). Therefore, we next assessed the effect of SphK inhibition on lymphocyte subsets in blood and lymphatic organs. Blood lymphocyte count remained unaltered in treated animals as compared to controls (data not shown). However, ABC294640 treatment led to a slight but significant decrease of CD4+ T and CD8+ T cell levels in blood, while the corresponding cell counts in lymph nodes and spleen were increased (Fig. 3A). In addition, lymph node and spleen CD4+ T cells (but not CD8+ T cells) were enriched in CD69, an early activation marker and a putative lymphatic retention signal (Fig. 3B). SphK inhibition did not noticeably influence total B cell (CD19) numbers in blood, lymph nodes and spleen (data not shown). In addition to lymphocyte count and subset pattern, we also addressed the influence of SphK inhibition on lymphocyte...
To this end, murine splenocytes from control and ABC294640-treated animals were stimulated with ConA (10.0 μg/ml) or PHA (5.0 μg/ml) and the proliferation rate of CD4+ T cells was determined by flow cytometry using a CFSE dilution assay. As shown in Figure 3C, both agonists potently stimulated proliferation of CD4+ T cells from control animals and this response was further enhanced in ABC294640-treated animals. In addition, the ConA- or PHA-induced production of IL-2 and IFN-γ – two cytokines released from activated CD4+ T cells – was bolstered up in splenocytes obtained from ABC294640-treated animals in comparison to control splenocytes (Fig. 3D).

SphK inhibition enhances dendritic cell activation

As T cell activation is controlled by antigen presenting cells, we next examined the effect of SphK inhibition on the function of macrophages and dendritic cells. To this purpose, peritoneal leukocytes were obtained from control and ABC294640-treated animals and the surface expression of co-stimulatory molecules (MHC class II, CD86) defining the subpopulation of activated antigen-presenting cells has been examined in cells positive for F4/80, CD11c, MHCII or CD86 and analysed by flow cytometry. Data are presented as fluorescence intensity. C) Peritoneal macrophages were established in culture, incubated for 24 h in the absence or presence of poly(I:C) (50.0 ng/ml) and TNFα, IL12p70 and IFNγ concentrations in the cell medium were determined using ELISA. D) Cytokine profile of pooled macrophage supernatants from six mice of control or ABC294640 treatment groups was determined semi-quantitatively by a RayBiotech cytokine array. Shown are arrays representative of one out of two determinations. 1. RANTES; 2. IL-4. E) Plasma levels of TNFα and IL12p70 were determined in each animal by ELISA. * – p<0.05 (ABC294640 vs. control).
IFNγ, which is produced by dendritic cells but not by macrophages. The extended analysis of cytokine and chemokine production using a semi-quantitative cytokine array additionally revealed that peritoneal cell supernatants from ABC294640 treated LDL-R−/− mice were enriched in RANTES and IL-4 (Fig. 4D). The modification of cytokine and chemokine production profile evoked in peritoneal cells by ABC294640 administration was paralleled by respective changes in plasma cytokine concentrations. As shown in Figure 4E, ABC294640 treatment of LDL-R−/− mice led to a marked elevation of the plasma levels of IL-12p70, while levels of TNFα remained unaltered.

SphK inhibition improves anti-adhesive properties of endothelial cells

As lymphocyte recruitment to arteriosclerotic lesions depends on the functional integrity of endothelium, we next investigated the effects of SphK inhibition on the anti-adhesive properties of endothelial cells. To this aim, bEnd.5 murine endothelial cells were pre-incubated for 1 h with 50.0 ng/ml of TNFα and adherent cells were determined using Western blot (Fig. 5A) and the adherence of calcein-loaded EL4 murine lymphocyte was observed under fluorescence microscope (B). Data are representative of three independent experiments. Right panel – quantification of EL4 lymphocyte adhesion to bEnd.3 cells. C) Western-type diet-fed LDL-R−/− mice were administered saline (n=12; Cont) or ABC294640 (1.5 mg/mouse; n=12; ABC) for 16 weeks. Plasma levels of soluble VCAM-1 (sVCAM-1), ICAM-1 (sICAM-1), and MCP-1 were determined in each animal by ELISA. D and E) Western-type diet-fed LDL-R−/− mice were administered saline (n=8; Cont) or ABC294640 (1.5 mg/mouse; n=8; ABC) for four weeks. The relative number of CD3+ cells in peritoneal cell suspension was determined in three animals 12 h after CCL19 injection (D). Tethering and adherence of perivitally labelled leukocytes to capillary vessel wall was examined after a single dose of LPS (5.0 mg/kg) in five animals using intravitral microscopy as described in Material and methods (E). Left panel: Original photographs of peritoneal arterioles. Arrows indicate fluorescently labelled adherent leukocytes. * – p<0.05; ** – p<0.01; *** – p<0.001 (ABC294640 vs. control).
gate, whether SphK inhibition affects the T cell capacity to egress the bloodstream and penetrate through endothelial barrier to extravascular space. As shown in Figure 5D, i.p. injection of CCL19 induced the mobilisation of CD3+ T cells to peritoneum in control LDL-R-/- mice, while this effect was substantially reduced in ABC294640-treated animals. Finally, leukocyte-endothelial interaction has been directly assessed in vivo in postcapillary venules. To this aim, leukocytes were perivitally labelled with fluorescence dye (CFDA) in control and ABC294640-treated LDL-R-/- mice, and cell rolling on and tethering to the vascular wall was monitored using intravital microscopy. Figure 5E demonstrates that ABC294640 administration substantially reduced leukocyte-endothelial interaction: the number of rollers was significantly reduced while the number of stickers tended to decrease in ABC294640 treated LDL-R-/- mice as compared to controls.

Discussion

S1P signalling is instrumental in the pathogenesis of several inflammatory diseases including ulcerating colitis, viral myocarditis, endotoxin-induced lung injury, or autoimmune encephalomyelitis (27–30). Recent studies showed that FYT720 – a synthetic S1P mimetic interacting with S1P receptor types present in vasculature – exerts anti-atherogenic effects in mouse models of disease (19, 20). However, the relevance of the endogenous S1P for the development of atherosclerosis has not been investigated to date. Therefore, it was the aim of the present study to assess the formation of the atherosclerotic lesions under conditions of SphK inhibition and reduced S1P formation. To suppress endogenous S1P synthesis we used ABC294640, a synthetic SK inhibitor that targets SphK2 at lower and SphK1 at higher concentrations, accumulates and reduces SphK activity in peripheral tissues, and shows anti-inflammatory and anti-tumor activities in murine models of diseases (21, 22, 26). In our hands, ABC294640 decreased plasma S1P levels by approximately 30–40%. This is comparable to the effect of SphK2 gene knock-out in mouse, in which both reduced plasma S1P levles and SphK activity were observed (31). However, neither absolute nor fractional lesion area was affected in LDL-R-/- mice treated with ABC294640.

The lowering effect of SphK inhibition on triglycerides is for the first time reported here and is rather unexpected in light of previous investigations, which failed to register any plasma lipid and lipoprotein alterations in mice treated with synthetic S1P mimetics (19, 20). However, increased generation of triglyceride-rich lipoproteins has been observed in mice with haematopoietic deficiency of S1P degrading enzyme, S1P lyase, which are characterised by substantially increased endogenous S1P levels in plasma (Bot M, unpublished observation). In addition, S1P was found to affect lipolysis in differentiated rat white adipocytes (32). Collectively, these observations point to the modulatory effect of S1P on triglyceride production and/or degradation, and mandate further research to clarify its role in the regulation of intermediary lipid metabolism.

In contrast to lipid metabolism, the profound regulatory impact of S1P on T cell distribution and function is well established (8, 9). Formation of increasing S1P concentration gradients between the lymphoid tissue and the adjacent blood is essential for the effective lymphocyte egress and recirculation, and the retention of T cells in spleen and lymph nodes was observed after S1PR1 elimination with genetic or pharmacologic approaches (33–35). Moreover, S1P negatively regulates cell surface expression of CD69 – an early activation marker and a putative retention signal within lymphoid organs (36). Functional studies revealed decreased proliferation and cytokine secretion in vitro by T cells exposed to S1P or in vivo after adoptive transfer of lymphocytes constitutively over-expressing S1PR (37–39). Consistent with these observations, the present study documents slight but significant redistribution of CD4+ T cells and CD8+ T cells from blood to spleen and lymph nodes that might be expected after reduction of the plasma S1P concentration and the plasma-lymphoid tissue S1P gradient. Moreover, the protracted inhibition of SphK activity led to the substantial enhancement of T cell activity as evidenced by the increased CD69 expression on the cell surface (predominantly on CD4+ T cells) as well as the proliferation and the cytokine production in response to ConA and PHA stimulation. The latter effects might arise in consequence of toning down the inhibitory influence exerted directly by S1P on T cell functions or by the increased activity of antigen presenting cells, which are vital for boosting T cell activity. Actually, the present study for the first time provides evidence for the increased surface expression of costimulatory molecules (CD86, MHC class II) reflecting the immunostimulatory activity on CD11c+ dendritic cells under conditions of chronic SphK inhibition. In addition, continuous treatment with SphK inhibitor was accompanied by increased plasma levels and/or production by peritoneal cells of IL-12p70 and IFNγ–cytokines synthesised in copious amounts by dendritic cells. Our in vivo findings corroborate results of several previous in vitro studies, which demonstrated inhibitory effects of both S1P and synthetic S1P mimetics on chemotactic responses and cytokine production by dendritic cells as well as on their capacity to serve as antigen-presenting cells (40–42). In particular, the increased production of IL-12p70 in SphK1 deficiency and its reversal by S1P agonists have been recently reported in dendritic cells differentiated from splenocytes (43). Taken together, the present data point to the persistent hyperactivation of dendritic cell-T cell axis under condition of chronic SphK inhibition and reduced endogenous S1P formation.

T cells are highly involved in the development of atherosclerosis. Both CD4+ T cells and T cell-derived cytokines are present throughout human and murine plaques and the adaptive immunity deficiency or the selective T cell deficiency both lead to reduced atherosclerosis in mice (44, 45). Similarly, dendritic cells are found in atherosclerotic lesions, where they are believed to orchestrate CD4+ T cells infiltration, priming, and clonal expansion (46). Given the crucial role of T cells and dendritic cells in atherosclerosis, the unaltered lesion burden in SphK inhibitor-treated animals is surprising and suggests that some anti-atherogenic mechanisms instigated by SphK inhibition might countervail pro-
Sphingosine kinase and atherosclerosis

Both pro- and antiatherogenic effects have been attributed to S1P and the exact role fulfilled by this compound in the pathogenesis of atherosclerosis remains a matter of lively debate. Most of the putative anti-atherogenic effects of S1P including stimulation of endothelial proliferation, migration, survival, and NO production as well as inhibition of pro-inflammatory activities of macrophages and lymphocytes are attributed to the ligation of G protein-coupled S1P receptors (4, 5). However, S1P acts also as a second messenger, which is formed by SphK downstream of extracellular signals such as pro-inflammatory cytokines and mediates direct intracellular effects via Ras, mitogen-activating protein kinase and/or Ca2+ currents (5, 10). The outcomes of intracellular S1P signalling, which encompass activation of the transcription factor nuclear factor-κB as well as expression of cytokines such as TNFα and adhesion-mediating molecules such as VCAM-1 and ICAM-1 in macrophages and endothelial cells, respectively, may be considered pro-atherogenic. Based on these findings the hypothesis has been previously put forward that extra- and intracellular S1P signalling play opposite roles in the atherosclerosis development (49, 50). The results of the present study, which demonstrate the simultaneous occurrence of pro- and anti-atherogenic effects under conditions of SphK inhibition and reduced S1P generation, lends for the first time experimental support to this hypothesis.

The present study has certain limitations. First, using ABC296640 as SphK inhibitor led to reduction of plasma S1P by approx. 30%, which may not be sufficient to fully expose its positive or negative influence on the progression of atherosclerosis. Second, as ABC294640 targets SphK1 and SphK2 at different concentrations, it cannot be excluded that both isoforms were not equipotently inhibited in course of the treatment. These limitations may additionally explain the neutral effects of ABC296640 on the development of atherosclerosis as opposed to clearly anti-atherogenic effects exerted by potent synthetic agonists of S1P receptors (19, 20). The present findings mandate further investigations of the effect of endogenous S1P on the development of atherosclerosis exploiting more potent and specific inhibitors or knock-outs of enzymes involved in S1P synthesis or degradation. In this context, it may be worth noticing that hematopoietic S1P lyase deficiency elevates endogenous S1P levels in plasma and decreases atherosclerotic lesion formation in LDL-R-/- mice (Bot M, unpublished observation).

In conclusion, treatment with SphK inhibitor leads to both pro- and anti-atherogenic effects in LDL-R-/- mice. As a consequence, SphK inhibition fails to affect atherosclerosis despite significant S1P reduction in plasma.

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Conflict of interests

Dr. Lynn Maines is Vice-President of Apogee Biotechnology Corp.

What is known about this topic?

- Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid and a constituent of high density lipoproteins that account for several anti-atherogenic effects of these lipoproteins in vivo.
- Synthetic mimetics of S1P such as FTY720 reduce atherosclerosis in animal models of disease (apoE-deficient mice, LDL-R-deficient mice).
- Anti-atherogenic effects of endogenously produced S1P have not been as yet investigated in vivo.

What does this paper add?

- We here for the first time examined development of atherosclerosis under conditions of sphingosine kinase inhibition and reduced generation of endogenous S1P.
- We demonstrate that inhibition of sphingosine kinase leads to both pro- and anti-atherogenic effects (via enhanced activation of dendritic cells and T-cells) and anti-atherogenic effects (via reduced activation of endothelial cells) in LDL-R-deficient mice. As a consequence, sphingosine kinase inhibition does not affect atherosclerotic lesion formation.
- This study highlights the complex interplay between S1P metabolism and signalling and diverse processes involved in the development of atherosclerosis.

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References


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