Chapter 4

The HIV Entry Inhibitor TAK-779 Attenuates Atherogenesis in LDL Receptor Deficient mice

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Abstract

HIV combination therapy using protease-inhibitors is associated with elevated plasma levels of atherogenic lipoproteins and increased risk for atherosclerosis. We investigated whether the HIV entry inhibitor TAK-779, affects lipoprotein levels and atherogenesis in LDL receptor deficient mice. TAK-779 is an antagonist for the chemokine receptors CCR5 and CXCR3, that are expressed on leukocytes, especially Th1 cells, and these receptors may be involved in recruitment of these cells to atherosclerotic plaques. TAK-779 treatment of LDLr−/− mice did not elevate the levels of atherogenic lipoproteins, whereas it dramatically reduced atherosclerosis in the aortic root and in the carotid arteries. The number of T cells in the plaque was reduced by 95%, concurrently with a 98% reduction in relative IFN-γ area. TAK-779 treated animals showed a decreased percentage of CD4+ and CD8+ T cells in peripheral blood and in mediastinal lymph nodes compared to control treated animals. TAK-779 not only suppresses HIV entry via blockade of CCR5 but also attenuates atherosclerotic lesion formation by blocking the influx of Th1 cells into the plaque. TAK-779 treatment may be especially beneficial for young HIV patients as they face lifelong treatment and this drug impairs atherogenesis.
Chapter 4

Introduction

Leukocyte recruitment into the vessel wall is a key step in atherosclerotic lesion formation and chemokines are known to regulate this process. Chemokines can be divided into four families including the CC receptors that bind CC chemokines, and the CXC receptors which bind CXC chemokines. CCR5 and CXCR3 are two chemokine receptors that are implicated in the migration of activated Th1 cells to the site of inflammation, and both receptors have been suggested as potential targets for the treatment of autoimmune-like diseases. A 32 base pair deletion in the CCR5 gene (CCR5Δ32) results in a non-functional receptor and individuals that are homozygous for this deletion are resistant to infection with HIV. Interestingly, it is also shown that this natural deficiency in CCR5 protects individuals from early myocardial infarction and severe coronary artery disease. The ligands for CCR5, RANTES and MIP-1α, have been detected in atherosclerotic plaques of both humans and mice. Furthermore, inhibition of CCR5/CXCR1 using the receptor antagonist met-RANTES attenuates atherosclerosis in LDLr deficient mice. TAK-779 is a non-peptide CCR5/CXCR3 antagonist, that was developed for the treatment of HIV infection by inhibiting HIV cell entry via CCR5. TAK-779 however has also some anti-immunogenic effects. It has been shown to block the influx of CCR5 and CXCR3 positive T cells into inflamed joints in an experimental model for arthritis.

Both CCR5 and CXCR3 are predominantly expressed on Th1 cells. As atherosclerosis is considered to be a Th1 mediated disease, treatment with TAK-779 could possibly attenuate atherogenesis by blocking the influx of Th1 cells into the atherosclerotic lesion. Both studies in humans and mice demonstrate an increase in atherosclerotic lesion formation and myocardial infarction in relation to the protease inhibitor treatment in HIV positive patients. TAK-779 could therefore have a dual function in these patients, as it not only blocks virus entry, but at the same time inhibits the severe side effects of their therapy.

In the present study we show that treatment of LDLr−/− mice with TAK-779 attenuates atherosclerotic lesion formation. TAK-779 treatment may therefore serve as a new convenient treatment of HIV infection and at the same time attenuate atherosclerotic lesion formation, in contrast to the now available combination therapy with protease inhibitors.
**Materials and methods**

*Animal experiments*

Female LDLr deficient (LDLr−/−) mice, 15 weeks old (n=10/group), were put on a Western-type diet containing 0.25% cholesterol and 15% cocoa butter two weeks before collar placement. Silastic collars (0.3 mm inside diameter, Dow Corning, Midland, USA) were placed around the carotid artery to induce atherosclerosis as described previously. Mice were treated immediately after collar placement with an injection of 150 μg TAK-779 in 100 μl of 5% mannitol (w/v) subcutaneously every other day during 6 weeks after which mice were anesthetized and subsequently sacrificed during tissue harvesting (5% mannitol was used as a control).

In a second experiment female LDLr−/− (mice n=10/group) were fed a Western type diet and TAK-779 treatment was started simultaneously. Treatment was performed for 6 weeks and hearts were excised and stored in Zinc Formal-Fixx and the aortic root was taken out for analysis.

*Histological analysis*

Cryosections were stained with hematoxilin (Sigma Diagnostics) and eosin (Merck diagnostics). Corresponding sections were stained with antibodies directed against a macrophage specific antigen (MOMA-2, polyclonal rat IgG2b, Research diagnostics); α-smooth muscle cell actin (monoclonal mouse IgG2a, clone 1A4, Sigma Diagnostics); IFN-γ (rat IgG1, clone XMG1.2, BD Pharmingen) or CD3 (molecular complex 17A2, BD Pharmingen) for 2 hours. As secondary antibodies (1 hour incubation) goat anti-rat IgG alkaline phosphatase conjugate (Nordic) with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as enzyme substrates, or biotinylated goat anti-rat polyclonal Ig (BD Pharmingen) was used in combination with streptABC complex (DAKO), with Nova Red as enzyme substrate (Vector Laboratories). Collagen was visualized by picrosirius red (Direct red 80) and lipids by Oil red O staining.

*Serum lipid levels*

Concentrations of serum cholesterol and triglycerides were determined using enzymatic colorimetric procedures (Roche/Hitachi, Mannheim, Germany). Precipath was used as a standard. Cholesterol distribution over the different lipoproteins was determined by fractionation of the serum using a FPLC system and subsequent cholesterol assay.

*Real time PCR assays*

Total RNA was isolated from spleens of the mice using GTC method. Purified RNA was DNase treated (DNase I, 5 units/μg of total RNA) and reverse transcribed (RevertAid M-MuLV reverse transcriptase) according to manufacturers protocol. Quantitative gene expression analysis (Primers,
appendix I) was performed on an ABI PRISM 7700 machine (Applied Biosystems, Foster City, CA) using SYBR Green technology.

Flow cytometry
Lympholite (Cedarlane Laboratories, Hornby, Ontario, Canada) was used to separate lymphocytes from whole blood and spleen. Cell suspensions from mediastinal lymph nodes, spleen and blood were incubated with 1% normal mouse serum in PBS and stained for surface markers (0.5 μg Ab/200,000 cells, BD Bioscience). All data were acquired on a FACSCalibur and 10,000 lymphocyte events were analyzed with CELLQuest software (BD Biosciences).

Figure 1. mRNA expression of inflammatory markers in the spleen in response to a Western type diet. mRNA was isolated from spleen using the GTC method and expression of different genes is expressed relative to 36B4 and HPRT, and subsequently related to the expression of mice on chow diet. An unpaired Student t test was applied to test whether mRNA levels were significantly different from the mRNA levels in chow fed animals (*p < 0.05, n=6 per time point).

Figure 2. Effect of TAK-779 treatment on serum lipid levels. LDLr−/− mice (n=6 per group) were put on a Western type diet and treated with 150 μg of TAK-779 s.c. per mouse every other day or control. At the indicated time points blood samples were taken and serum cholesterol (2A) and triglyceride (2B) levels were determined. Distribution of cholesterol over lipoproteins was determined using FPLC. 30 μl aliquots of serum of individual mice were loaded on a Pharmacia SMART column and 24 fractions were collected. Cholesterol concentrations were determined at chow (2C) and Western type diet (2D) for TAK-779 treated and control mice.
Results

mRNA expression in spleen of CCR5, CXCR3 and their ligands
Atherosclerosis is considered to be an inflammatory disease, primarily characterized by a Th1 mediated inflammatory reaction. CCR5 and CXCR3 are chemokine receptors that are mainly expressed on Th1 cells. The spleen plays a central role in the immune system and is exposed to circulating antigens associated with atherosclerosis such as oxidized LDL. We examined the mRNA expression of CCR5 and CXCR3 and their ligands in the spleen during the process of atherosclerosis in LDLr-/- mice on Western type diet (Figure 1).

After 6 weeks of diet, CCR5 relative mRNA expression in the spleen is significantly upregulated 2.4 fold (1.0±0.06 vs. 2.4±0.21, p=0.01). The expression of CXCR3 is not differentially regulated during the first 6 weeks of feeding a Western type diet and atherosclerotic lesion formation. Both CCR5 and CXCR3 are downregulated in the spleen in later stages of atherosclerotic lesion formation in the used model (> 9 weeks of Western Type diet). The expression of established ligands for CCR5 was also monitored. RANTES and MIP-1α are both significantly upregulated after 6 weeks of Western type diet. This coincides with the regulation pattern of CCR5 and MCP-1. CXCL10, a ligand specific for CXCR3 is downregulated after 9 weeks of Western type diet and showed no regulatory similarity with the other ligands.

TAK-779 reduces atherogenesis in LDLr-/- mice without altering cholesterol levels
Two different experiments were performed to study the effect of TAK-779 on atherogenesis.

After two weeks of Western type diet LDLr-/- mice were equipped with collars around both carotid arteries. Subsequently, the mice were treated with TAK-779 or control treated for 6 weeks. Yang et al. showed that administration of 150 μg per mouse every other day was sufficient to induce the maximum blocking effect. No difference in cholesterol levels and lipoprotein concentration could be observed between the control and TAK-779 treated group (Figure 2). Figure 3 shows representative sections of TAK-779 treated (figure 3D) and control treated (figure 3E) mice. Lesion size significantly decreased by 68% upon treatment with TAK-779 (18384±3370 μm² vs. 5926±1842 μm², p<0.004) (figure 3A), intima media ratio decreased by 49% (0.46±0.07 vs. 0.26±0.06) (figure 3B), and also the relevant clinical parameter, intima lumen ratio, decreased significantly by 56% (0.26±0.04 vs. 0.11±0.03, p=0.01) (figure 3C).

The general composition of the carotid lesions of TAK-779 treated mice was not altered compared to control treated mice (figure 4). The relative MOMA-2 stained area was comparable in control (4B) and TAK-779 treated animals (4C), (0.265 ± 0.05 vs. 0.252 ± 0.06, p=0.88). We visualized the collagen content of the plaque by picrosirius red staining. No difference was observed between control (4E) and treated mice (4F) (0.095 ± 0.03 vs. 0.101 ± 0.03, p=0.69). Staining for α-
actin, specific for smooth muscle cells (ASMA) is shown in on line figure 4H (control) and 4I (treated). TAK-779 treatment did not affect relative ASMA area (0.084 ± 0.016 vs. 0.078 ± 0.011).

In a second experimental setup, Western type diet and TAK-779 treatment were started simultaneously and 8 weeks later LDLr−/− mice were sacrificed. Atherosclerosis in the aortic valves was decreased by 40% in TAK-779 treated mice compared to control mice without altering relative macrophage content (Figure 5).

Figure 3. Effect of TAK-779 treatment on plaque formation in a collar induced carotid artery atherosclerosis model. Cross-sectional carotid plaque area (3A), intima/ lumen ratio (3B), and intima/ media ratio (3C) following a treatment with 150 μg of TAK-779 in 100 μl of 5% mannitol injection fluid s.c. every other day during 6 weeks. A marked and significant decrease was seen for all three parameters. Lower panel gives representative pictures of the carotid artery of control (3D) and TAK-779 treated (3E) LDLr−/− mice. Error bars represent SEM, n = 10 per group, (**= p<0.005, *=p<0.05, Mann Whitney test).

TAK-779 treatment impairs the influx of Th1 T cells to the atherosclerotic plaque

Both CCR5 and CXCR3 are expressed predominantly on Th1 cells and both receptors are implicated in the migration of these cells to their site of action and treatment with TAK-779 may block the migration of Th1 cells into the atherosclerotic plaque. We performed a staining for CD3 (figure 6A), a general T cell marker and scored for positive cells in both the plaque and the adventitia. A vast and significant decrease is observed in the number of T cells in the plaque and adventitia of TAK-779 treated mice compared to control (plaque 0.53 ± 0.31 vs. 0.03 ± 0.03, p=0.04, adventitia 4.98 ± 0.63 vs. 2.01 ± 0.38, p=0.0007). In order to investigate whether the reduction of T cells resulted in a decreased expression of Th1 specific markers we stained sections of control (6D) and TAK-779 treated
mice (6E) for IFN-γ. A significant reduction of 98% was observed in the IFN-γ positive area in TAK-779 treated animals compared to control (0.55% ± 0.21% vs. 0.01% ± 0.0004%, p=0.013).

Figure 4. General plaque composition is not altered by TAK-779 treatment. Plaque composition of TAK-779 treated and control treated mice was determined using a macrophage specific antibody (MOMA-2) (figure 4A-C), picosirius red staining to visualize collagen (figure 4D-F) and smooth muscle cell specific staining (α-SM acin) (figure 4G-I). Representative sections are shown for each group. No significant effects could be observed between control and treated animals (n=10 per group).

TAK-779 reduces T cell counts in LDLr⁻/⁻ mice
Both CCR5 and CXCR3 are reported to be involved in T cell migration to inflamed tissue3,14. To investigate whether T cell numbers were affected during atherogenesis in TAK-779 treated mice versus control treated mice, lymphocytes were harvested from blood, from mediastinal lymph nodes, which drain from the aortic arch, and from spleen 6 weeks after the start of Western type diet. Cells were stained for CD4 and CD8, gated for lymphocytes in the FSC/SSC plot, and the percentages of CD4⁺ and CD8⁺ T cells were determined by flow cytometric analysis. Figure 7A shows representative dot plots for control treated mice (upper three panels) and TAK-779 treated mice (lower three panels) on a Western type diet. A vast decrease in the percentage of CD4⁺ T cells in peripheral blood (21.6±3.11 % vs. 10.46±1.57 %, p=0.02) and a modest decrease in lymph nodes (35.3±2.5 % vs. 26.9±4 %, p=0.14) was observed for the TAK-779 treated group.
Figure 5. TAK-779 treatment reduces plaque formation in the aortic leaflet area.
Representative photomicrographs of oil red O stained cross sections of the aortic root of control (5A) and TAK-779 treated mice (5B) are shown. A significant reduction in plaque size was found as compared to control (p=0.001, n=8 per group) (5C). Relative macrophage staining of control (5D) and treated mice (5E) is visualized using a monoclonal antibody specific for macrophages (MOMA-2). No difference in the percentage MOMA positive area is observed between control and treated mice (5F).

Figure 6. Migration of Th1 cells in TAK-779 treated animals is reduced compared to control treated animals.
Sections of collar induced lesions were stained for T cells using an anti-CD3 antibody (6A). The number of positive cells in both plaque and adventitia was quantified. A significant reduction of the number of CD3 positive cells was observed both in the plaque (6B) as in the adventitia (6C) of TAK-779 treated animals compared to control (n=10 per group). Representative sections with IFN-γ staining are shown for control (6D) and treated mice (6E). A significant reduction in the relative IFN-γ area is observed after TAK-779 treatment compared to control (6F).
In contrast, the CD4+ population was significantly increased in the spleen of the TAK-779 treated mice (9.6 ± 1.22 % vs. 13.5 ± 0.9 %, p=0.03) (figure 7B). An even more pronounced decrease was observed in the CD8+ T cell population. In the circulation the percentage CD8+ T cells decreased from 10.2±1.8 % in the control group to 3.8±0.7 % in the treated group (p=0.01). In the mediastinal lymph nodes draining from the aortic arch, a reduction of 34% in the number of CD8+ cells was observed (21.4±0.52 % vs. 14.6±2.0 %, p=0.02) (figure 7C).

No significant differences in total white blood cell counts were observed between control and treated mice.

**Inflammatory status in the spleen after TAK-779 treatment**

After 6 weeks of simultaneous Western type diet feeding and TAK-779 treatment, the mRNA expression of CCR5 and CXCR3 was determined to evaluate the expression of the two chemokine receptors that were specifically blocked by TAK-779 (figure 8). CCR5 expression was 1.9-fold increased in TAK-779 treated mice in comparison with control mice (p=0.02), whereas the expression of CXCR3 remained unchanged. The expression of CCR2, a chemokine receptor involved in the migration of macrophages and T cells and also implicated in atherosclerosis, is highly elevated in the spleen upon TAK-779 treatment. The expression of endogenous ligands for these receptors was also evaluated. The splenic mRNA levels for MIP-1α, RANTES and CXCL10, which bind to CCR5 and CXCR3 respectively, were not affected by TAK-779 treatment, while the expression of monocyte chemotactic protein-1 (MCP-1), a ligand for CCR2, was significantly upregulated 8.1-fold. We also determined the expression of some cytokines that are important in the Th1/Th2 balance. IL-12, a general Th1 stimulatory interleukin is 2.8-fold upregulated in the spleen of TAK-779 treated mice as compared to control treated mice, while the Th2 interleukin IL-4 is 3.2-fold upregulated. This results in an unaffected Th1/Th2 balance upon TAK-779 treatment, and the observed increase in splenic cytokine expression might be explained by the increased number of CD4+ T cells in spleen (figure 8).

**Discussion**

Infiltration of mononuclear cells into the vessel wall is an important hallmark of atherosclerosis. It has become clear that next to macrophages, T lymphocytes are present in the atherosclerotic plaque and exert a significant role in plaque progression. The majority of these T cells is CD4 positive. Activation of CD4+ T cells via MHC class II on antigen presenting cells results in the release of cytokines and the expression of several surface markers. Activated CD4+ T cells can be divided in 2 distinct categories based on their phenotype. Th1 cells secrete IFN-γ, IL-2 and TNF-α. Th2 cells produce IL-4, IL-5 and IL-13 leading to antibody production and the elimination of extracellular pathogens.
The HIV entry inhibitor TAK-779 is a CCR5 and CXCR3 antagonist in mice and several studies have shown that TAK-779 reduces autoimmune responses by interfering with the migration of Th1 cells to the inflamed tissue. We argue that TAK-779 may therefore also affect atherogenesis in a similar way. As the current treatment for HIV patients is associated with increased atherogenic lipoprotein concentration and accelerated atherosclerosis, an anti-HIV drug that reduces atherosclerosis would be of great importance. As atherosclerosis is considered to be a Th1 mediated disease, modulation of the activation and migration of Th1 T cells provides an attractive therapeutic possibility.

Figure 7. Percentages of CD4 and CD8 positive T cells in mediastinal lymph nodes (MLN), blood, and spleen. Mononuclear cell suspensions of draining lymph nodes, spleen and blood were isolated from control mice and mice following a treatment with 150 μg of TAK-779 in 100 μl of 5% mannitol injection fluid s.c. every other day during 6 weeks. Representative dot plots are shown in figure 7A. Cells were stained for CD4 (7B), and CD8 (7C). Results represent the mean percentage of positive cells ± SEM from 5 individual mice (**=P<0.005, *=P<0.05, Students T test). A decrease is seen in the percentage CD4 and CD8 positive T cells in DLN and blood in the TAK-779 treated group (White bars) compared to control (Black bars). In the spleen, the percentage of CD4 positive cells is increased in the treated group.
Figure 8. Spleen mRNA expression of inflammatory markers after TAK-779 treatment. mRNA expression of different genes in the spleen of TAK-779 treated mice (8 weeks) is expressed relative to 36B4 and HPRT, and subsequently related to the expression in control mice. White bars represent control mice, black bars represent TAK-779 treated mice. An unpaired Student t test was applied to test if mRNA levels were significantly different from the mRNA levels in chow fed animals (n=10 per group) (*P<0.05).

CCR5 and CXCR3 are chemokine receptors that are both primarily expressed on Th1 T cells. A putative role for CCR5 and CXCR3 in atherogenesis is implicated by studies in which a mutation in the CCR5 sequence (Δ32 mutation) was found to be associated with a reduced incidence in myocardial infarction and severe coronary artery disease. Furthermore, antagonizing CCR5 using met-RANTES also resulted in reduced atherosclerotic lesion formation.

In the present study we firstly examined the expression of CCR5 and CXCR3 in the spleen of LDLr−/− mice during Western type diet feeding. It appeared that CXCR3 was not differentially regulated in the spleen during the first 6 weeks of atherogenic diet, whereas CCR5 showed a significant upregulation in this period. The known ligands of CCR5, RANTES and MIP-1α have been detected in atherosclerotic plaques, and these molecules were also upregulated at 6 weeks of Western type diet feeding in the spleen. No distinct regulatory pattern could be observed for the ligands of CXCR3, interferon gamma inducible protein-10 (IP-10) and CXCL10. These findings suggest that CCR5 may have a more
prominent role in the response of the immune system towards elevated cholesterol levels and atherogenesis.

In our studies the synthetic low molecular weight CCR5/CXCR3 antagonist TAK-779 was used to assess the role of these receptors in the process of atherosclerotic lesion formation. Treatment with this potential HIV entry inhibitor appeared to reduce plaque formation in the carotid artery with 68% and lesion formation in the aortic root with 40%. These findings are in line with a study by Veillard et al., who used met-RANTES to antagonize CCR5 and CCR-1, and observed a decrease in lesion formation as a result of reduced infiltration of mononuclear cells into the lesion\(^{10}\). In contrast with these observations, Kuziel et al. have published that disruption of the mouse CCR5 gene has no effect on early lesion formation in apoE deficient mice\(^{28}\). As these mice lack CCR5 throughout their development, it may be that impaired T cell migration due to CCR5 disruption is counterbalanced by a compensatory chemokine receptor mediated mechanism such as CCR-2/MCP-1.

The expression of CCR5 and CXCR3 has been well characterized on CD4 \(^{+}\) T cells and both chemokine receptors are preferentially expressed on the Th1 cell subset\(^{25,26}\). Limited expression of CCR5 is also reported on CD8 \(^{+}\) cells, human vascular smooth muscle cells and macrophages\(^{30,31}\). Our data show that the relative macrophage content of the atherosclerotic plaques did not differ between TAK-779 treated and control treated mice. This implies that migration of macrophages into the intima is not impaired by CCR5/CXCR3 blockade by TAK-779. Therefore the decreased lesion formation is more likely to be explained by the diminished migration of T cells from the spleen towards the sites of atherogenesis. This is further supported by the finding that the number of CD3 positive T cells in the plaque as well as in the adventitia is significantly decreased in TAK-779 treated animals. We also observed that the relative T cell counts in the blood and draining lymph nodes for both CD4 \(^{+}\) and CD8 \(^{+}\) T cells are significantly decreased in TAK-779 treated animals. Simultaneously, the percentage of CD4 \(^{+}\) T cells in the spleen is increased, suggesting that these T cells are retained in the spleen upon TAK-779 treatment.

Veillard et al. described that antagonizing CCR1 and CCR5 with met-RANTES results in decreased macrophage and T cell influx into the atherosclerotic lesion, in contrast to antagonizing CCR5/CXCR3 with TAK-779 which only affects T cell influx. This difference may be explained by the fact that met-RANTES antagonizes CCR1 which is expressed at high levels on macrophages, in contrast to CCR5 and CXCR3. TAK-779 is therefore more selective in antagonizing Th1 T cells, leaving macrophage recruitment mostly intact. We furthermore used an in vitro setup to test whether TAK-779 had any effect on macrophage adhesion to LPS stimulated endothelial cells. No dose-effect relationship for TAK-779 incubation of macrophages on endothelial adhesion was observed (data not shown). This further strengthens our findings that there is no major effect on macrophage adhesion and infiltration in TAK-779 treated animals.
It has been shown that local T cells secrete cytokines, which in turn activate and attract other inflammatory cells and therefore stimulate the inflammatory process. As CCR5 and CXCR3 are most strongly expressed on Th1 cells it is expected that mice treated with TAK-779 show smaller amounts of Th1 cytokines in the atherosclerotic plaque. We stained atherosclerotic plaque sections of control and treated mice for IFN-γ, a Th1 marker. In plaques of treated animals, the relative IFN-γ area was decreased by 98%, indicating that IFN-γ production by Th1 cells was almost completely absent upon TAK-779 treatment.

Our data show that TAK-779 blockade resulted in a marked upregulation of CCR2/MCP-1 in the spleen, which coincides with an upregulation of CCR5 in the spleen in comparison to control mice. This may be explained by the fact that CCR2 and CCR5 coexist in the same lipid raft domain and both receptors share a migratory function. Inhibition of CCR5 is then compensated by an upregulation of CCR2 to sustain migratory capacity.

Baba et al. have shown that TAK-779 has some affinity for CCR2b in humans, but this is 100 fold lower in comparison with CCR5. In addition, TAK-779 is not able to bind human CXCR3. Therefore it is likely that TAK-779 exerts its action by inhibiting CCR5 function in humans. We observed similar effects in our study in mice, as the expression of CXCR3 in spleen of mice treated with TAK-779 is not increased, in contrast to the expression of CCR5, thus implying a dominant role for CCR5 as target of TAK-779.

The increase in individual expression of the different chemokine receptors, combined with the unaltered Th1/Th2 ratio supports the theory that TAK-779 targets CCR5 and impairs T cell migration to the site of action, the atherosclerotic plaque, and does not alter the general inflammatory status of the animals.

Combination therapy which is commonly used to treat HIV patients has been shown to lead to elevated serum levels of the atherogenic lipoproteins LDL and VLDL, which result in a higher incidence in cardiovascular disease. We now show that TAK-779, a novel HIV entry inhibitor, does not elevate serum total cholesterol levels or triglyceride levels. More important, no increase in atherogenic lipoprotein levels was observed in the blood of TAK-779 treated animals, and a decrease was observed in atherosclerotic lesion formation. This is in contrast with patients and animal models treated with protease inhibitors.

In summary, the use of HIV entry inhibitors in the long-term treatment of HIV would be preferential over the now used combination therapy. It achieves long-term suppression of the virus and as shown in this study, retained atherosclerotic lesion formation by blocking the influx of Th1 cells in the atherosclerotic plaque. This finding is primarily beneficial for young HIV patients, as they face a livelong treatment and are currently confronted with the severe side effect of heart disease.
References


