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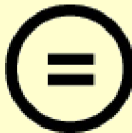

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Dual CCR2/5 antagonist attenuates obesity-induced insulin resistance by regulating macrophage recruitment and M1/M2 status

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Dual CCR2/5 antagonist attenuates obesity-induced insulin resistance by regulating macrophage recruitment and M1/M2 status

Directed by Professor Choon Hee Chung

A dissertation
Submitted to the Department of Medicine
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Ji Hye Huh

December 2017

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2017년 12월

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ABSTRACT

Dual CCR2/5 antagonist attenuates obesity-induced insulin resistance by regulating macrophage recruitment and M1/M2 status

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Adipose tissue inflammation induced by macrophage infiltration through the C-C motif chemokine receptor (CCR) 2 or CCR5 pathway has a pivotal role in obesity related disease and insulin resistance. Here we evaluated the effect of PF4178903, a dual CCR2/CCR5 antagonist, on the obesity and insulin resistance. 40 male C57BL/6J mice were divided into four groups as follows: 1) regular diet

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(RD); 2) RD with PF4178903; 3) high fat diet (HFD), and 4) HFD with PF4178903. All mice were sacrificed 12 weeks after the beginning of the experiment. Biochemical analyses and adipose tissue examinations were performed. After treatment with PF4178903, both body weight and adipocyte size in white adipose tissue (WAT) were decreased in HFD-fed mice. Furthermore, PF4178903 treatment reduced adipose tissue macrophages (ATMs) and lowered serum pro-inflammatory cytokines in HFD-fed mice. PF4178903 treatment significantly improved HFD-induced insulin resistance and glucose intolerance. Fluorescence-activated cell sorter (FACS) analysis revealed that PF4178903 treatment reduced the CD8+ T cell fraction in WAT of HFD mice. PF4178903 treatment reduced M1-polarized macrophages while inducing an M2-dominant shift in macrophages within WAT in HFD-fed mice. Dual CCR2/CCR5 antagonism ameliorates insulin resistance and inflammation in obesity by regulating ATMs recruitment and polarization in WAT.

Key Words: chemokines, obesity, insulin resistance, inflammation, macrophage

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I. INTRODUCTION

Obesity-induced chronic inflammation is critical in the pathogenesis of insulin resistance and subsequently leads to diabetes, cardiovascular disease, and several types of cancer.[1, 2] Increasing evidence supports the idea that obesity-induced inflammation is mediated primarily by immune cells, such as macrophages and T lymphocytes, in metabolic tissues. Specifically, adipose tissue macrophages (ATMs) are considered to play an essential role in chronic inflammation development during obesity.[3] As a source of pro-inflammatory cytokines [e.g. tumor necrosis factor (TNF)-α and interleukin (IL)-6], ATMs can block insulin action in adipocytes and may serve as a potential link between inflammation and insulin resistance.[4, 5] Furthermore, recent studies have categorized ATMs according to the M1/M2 system and the general consensus at present is that obesity induces the exclusive polarization of ATMs from M2 phenotypes (or "alternatively activated" non-inflammatory macrophages) into M1 phenotypes (or "classically activated" pro-inflammatory macrophages).[6] Thus, both recruitment and M1/M2 status of ATMs are pivotal in obesity-induced inflammation and insulin resistance.

Chemokines are small proteins that direct the trafficking of immune cells to sites of inflammation.[7] Advances in obesity research have led to the recognition

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of the role of chemokine–chemokine receptor interactions in ATMs accumulation. Increasing evidence implicates CCR2/CCR5 and their ligands [including C-C chemokine ligand type 2 (CCL2), also known as monocyte chemotactic protein-1 (MCP-1)] and type 5 [CCL5, also known as Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES)]) in the pathogenesis of insulin resistance through the promotion of macrophage recruitment and tissue infiltration.[8, 9] Thus, both CCR2 and CCR5 may be attractive targets for obesity induced insulin resistance and its comorbidities.

Although several previous studies have demonstrated that a CCR2 antagonist improved obesity induced insulin resistance,[10, 11] there has been no study investigating the effect of a dual CCR2/CCR5 antagonist on obesity induced chronic inflammation and insulin resistance. PF4178903 is a potent, selective, and orally bioavailable dual CCR2 and CCR5 antagonist (CCR2 and CCR5 binding IC50 values = 3.0 and 5.3 nM, respectively).[12] Therefore, we investigated whether a dual CCR2/CCR5 antagonist, PF4178903, attenuates inflammation and insulin resistance in obesity by regulating both adipose macrophage recruitment and M1/M2 status.

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II. MATERIALS AND METHODS

1. Mice

Eight-week-old 40 male C57BL/6J mice (Charles River Laboratories, Yokohama, Japan) were divided into four groups and fed for 12 weeks as follows: 1) regular diet (RD) (N=10) ; 2) RD with PF4178903 (10 mg/kg/day) (N=10); 3) high fat diet (HFD, consisting of 60% fat, catalogue No. D12492, Research Diets, NJ, USA) (N=10); and 4) HFD with PF4178903 (10 mg/kg/day) (N=10). PF4178903 was obtained from Pfizer and PF4178903 treatment was given by oral gavage once daily. The animals were maintained in an animal facility at 20-22 ℃ with 40-60% relative humidity and a 12 h / 12 h (light/dark) cycle for at least 7 days before the experiment. The amount of HFD was adjusted according to changes in body weight for each mouse. Food intake and body weight were measured periodically. All extracted tissues were immediately frozen in liquid nitrogen and stored at -80℃ until analysis. All experiments were performed in accordance with the National Institutes of Health guidelines and with the approval of the Yonsei University Institutional Animal Care and Use Committee (YWC-160804-1, Wonju, Korea).

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2. Glucose tolerance tests

We performed glucose tolerance tests to determine the glucose intolerance state of each group of mice at the 12th week of the study. Twelve-hour fasting blood glucose levels were analyzed with the Auto-Chek (Diatech, Seoul, Korea) from tail vein blood. Glucose (1.0 g/kg) was administered by intraperitoneal injection for glucose tolerance tests, and insulin solution (0.75 U/kg body weight, Humulin R; Eli Lilly, Indianapolis, IN, USA) was administered by intravenous injection for insulin tolerance tests.

3. Immunofluorescences

The paraffin-embedded epididymal white adipose tissue sections were stained with the combinations of anti-F4/80 (Abcam, Cambridge, U.K.), anti-CCR5 and anti-CCR2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Alexa Fluor 647 donkey anti-rat IgG (Invitrogen) was used as a secondary antibody to detect F4/80+ cells. Simultaneously, Alexa Fluor 488 mouse anti-goat IgG (Invitrogen) was used to detect CCR5+ cells and Alexa Fluor 488 goat anti-mouse IgG (Invitrogen) was used to detect CCR2+ cells.

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4. **Hematoxylin and eosin staining (H&E) and adipocyte cell size assess ment**

Paraffin sections of formalin-fixed epididymal white adipose tissue were stained with H&E for morphological evaluation. The area of each adipocyte was quantified using Image J software (National Institutes of Health, Bethesda, MD).

5. **Isolation of adipose tissue-infiltrating cells**

Mouse epididymal adipose tissue was minced and digested at 37℃ with shaking in PBS supplemented with 1mg/ml collagenase type 2 (Sigma) for 40 minutes. The digested tissue was filtered through a 100 µm nylon mesh to remove undigested tissue and centrifuged at 1,500 rpm for 5 min. The stromal vascular fraction (SVF) was obtained from the resulting pellet.

6. **Flow cytometry**

Immune cells isolated from epididymal adipose tissues were incubated with FcBlock (BD Biosciences, San Jose, CA, USA) in the dark at 4℃ on a bidirectional shaker for 30 min, and triple-stained with PEcy7-conjugated anti-

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F4/80, PE-conjugated anti-CD11c, FITC-conjugated anti-CD206 or PE-conjugated anti-CD3, FITC-conjugated anti-CD4 and PEcy7-conjugated anti-CD8 antibodies (eBioscience, San Diego, USA). The cells were then washed with fluorescence-activated cell sorting buffer and quantitated with a FACSVerse analyzer (BD Biosciences) with BD FACSuite (BD Biosciences). Cytometric bead assay (CBA) for mouse inflammation (BD Biosciences) was performed following the recommended assay procedure. Supernatants from macrophage infections were used for CBAs.

7. Enzyme-linked immunosorbent assay (ELISA)

The mice were euthanized after an 8-h fast, and blood was collected through cardiac puncture. Serum cholesterol, triglyceride, glucose, and insulin levels were measured levels at time of sacrifice. Serum glucose, total cholesterol and triglyceride concentrations were determined using commercially available enzymatic assay kits (Asan Pharmacology, Seoul, Korea). Serum insulin levels were measured using an ultrasensitive mouse insulin ELISA kit (Shibayagi, Gunma, Japan), and the serum adiponectin level was measured using an ELISA kit (AdipoGen Inc., Seoul, Korea). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as fasting serum insulin (mU/L) × fasting

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plasma glucose (mg/dl)/405.

8. Western blot analysis

The epididymal adipose tissue of each mouse was dissected and immediately frozen in liquid nitrogen. Lipids were removed by centrifugation at 10,000 × *g* for 20 min. Total protein (50 μg) was subjected to Western blot using various polyclonal antibodies. The intensities of the bands were measured with an Image J Analyzer (Biocompare, San Francisco, CA, USA).

9. Cell culture

RAW264.7 macrophages (ATCC, USA) were grown at 37℃ in 5% CO_2 in RPMI1640 medium (Gibco BRL, NY, USA) containing 10% fetal calf serum, 10ml/L penicillin streptomycin (Invitrogen, Carlsbad, CA) and medium was changed every 2 days. Free fatty acids (oleic acid, 250 μM, Sigma-Aldrich) were dissolved in ethanol containing bovine serum albumin (BSA, 50 μM) and conjugated with BSA at a 10:1 molar ratio before use.

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10. Isolation of adipose tissue-conditioned medium

Mesenteric adipose tissue was isolated from C57BL/6 male mice (8 weeks old; Koatech Ltd., Gyeonggi-do, Korea) fed HFD for 2 months. All subsequent procedures were performed in a laminar-flow hood. The adipose tissue was minced into fragments less than 10 mg in weight and cultured. Briefly, 500 mg of tissue was seeded in 10 ml of serum-free medium in the wells of a 100mm dish, and the dish was placed in a humidified incubator at 37℃ and 5% CO_2 for 3 days.

11. Migration assay

Cell migration was evaluated in a multi-well micro chemotaxis chamber (Neuro Probe). RAW264.7 macrophages cells were suspended in either RPMI 1640, adipose tissue conditioned medium (ATCM), or CCL2 and CCL4 at a concentration of 1×10^4 cells/ml, and then 60 ul of each solution was placed in the upper layer of a 96-well chamber membrane whose lower well contained or did not contain PF4178903. Following incubation at 37℃ for 4 h, the cells that had not migrated were removed, and the cells that had migrated across the filter were fixed, stained with Diff-Quik (International Reagent Corp, Darmstadt, Germany),

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and counted (n=4).

12. Statistical analyses

All data are presented as mean ± SEM. Statistical analysis was performed using a one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons, using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

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III. RESULTS

1. Obesity increases both CCR2 and CCR5 expression in adipose tissue macropahges

To investigate the roles of CCR2 and CCR5 in obesity, we analyzed CCR2 and CCR5 expression in epididymal white adipose tissue using immunofluorescence staining. Immunofluorescence analysis of white adipose tissue revealed that both CCR2 and CCR5 were highly expressed by F4/80+ macrophages in crown-like structures of HFD-fed obese mice compared to in RD-fed control mice (Figure 1a). ATCM from HFD-fed mice induced both CCR2 and CCR5 expression in RAW264.7 macrophage cells (Figure 1b). These findings indicate that obesity induce both CCR2 and CCR5 expression in adipose tissue macrophages.

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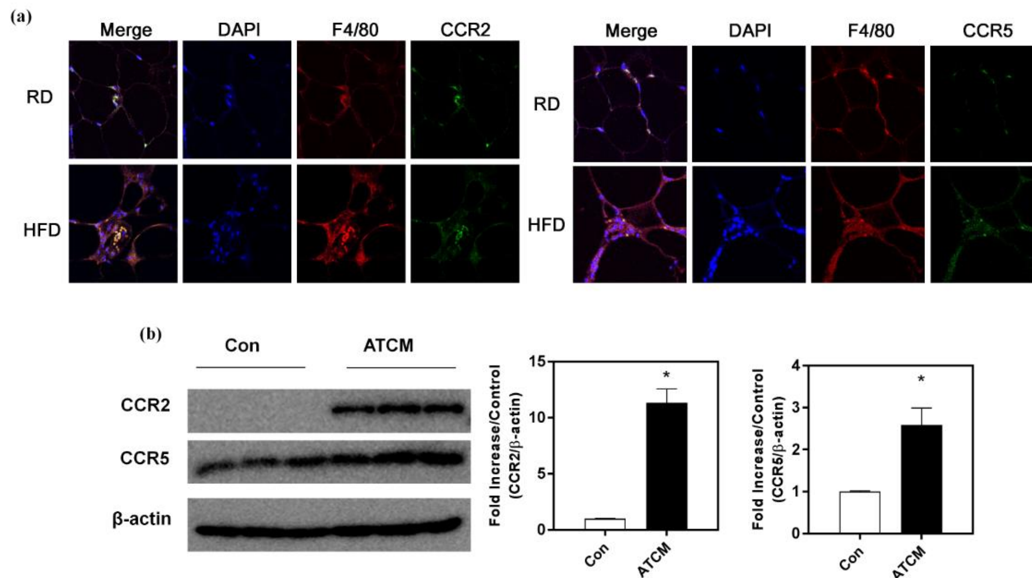

Figure 1. Obesity increases both CCR2 and CCR5 expression in adipose tissue macrophages. (a) Immunofluorescence staining for F4/80 (red) and CCR2/ CCR5 (green) in white adipose tissue from regular diet (RD) and high fat diet (HFD) wild type (WT) mice. Original magnification is x400 (scale bar = 50 μm). (b) Protein levels of CCR2 and CCR5 in RAW264.7 macrophage cells as determined by Western blot. Cropped membrane was used in Western blot. The band intensities were measured using Image J.

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2. PF4178903 inhibits CCL2/CCL4-induced migration of macrophage in vitro

We next examined the effect of PF4178903 treatment on Raw 264.7 macrophage migration using a trans-well system. RAW264.7 macrophage cells were treated with CCL2 (as a ligand of CCR2), CCL4 (as a ligand of CCR5) or ATCM from HFD-fed mice. As shown in Figure 2a-b, whereas CCL2, CCL4 and ATCM significantly induced macrophage migration, PF4178903 treatment markedly inhibited it. This finding indicates that treatment with PF4178903 can inhibit the macrophage migration capacity by induced obesity related factors *in vitro*.

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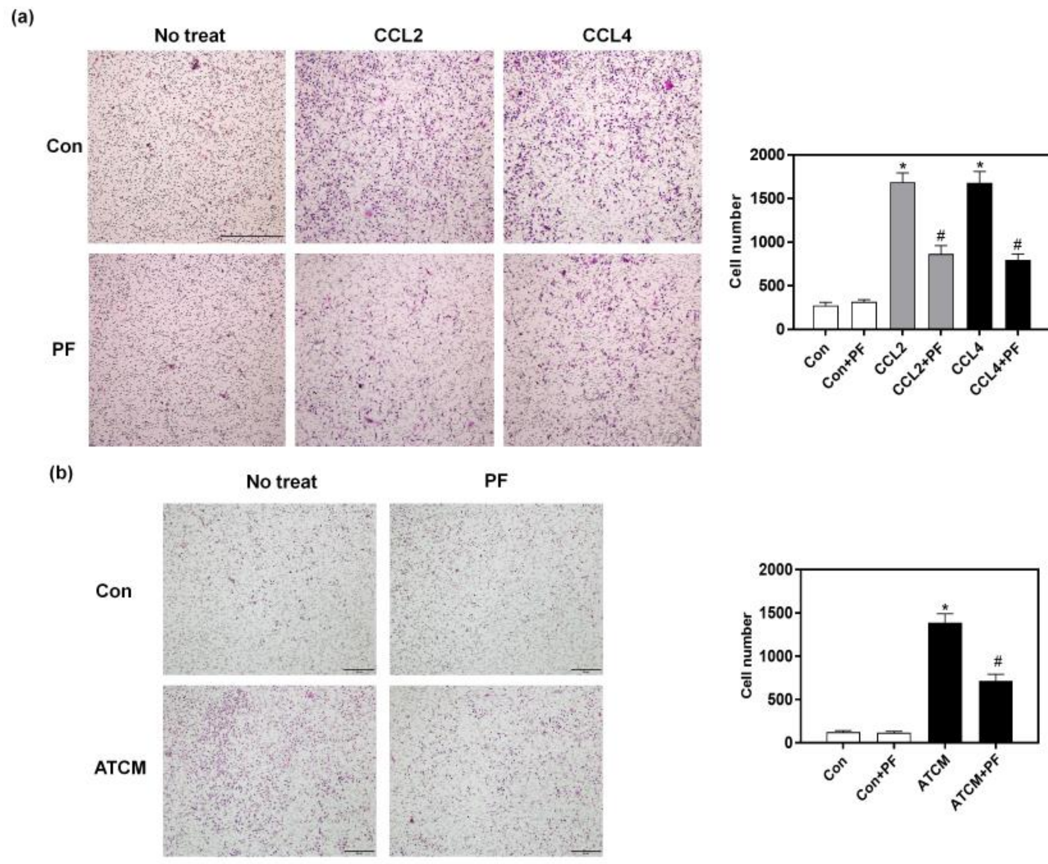

Figure 2. PF4178903 inhibits chemokines and adipose tissue conditioned medium-induced macrophage migration (a) RAW264.7 macrophages cells were treated with CCL2 (10 ng/ml) or CCL4 (10 ng/ml) in the presence and absence of PF4178903 (10μM/ml) for 24h (n=4). (b) RAW264.7 macrophages cells were treated with adipose tissue conditioned medium (ATCM) from HFD-fed mice in the presence and absence of PF4178903 (10μM/ml) for 24h (n=4)

The number of transmigrated peritoneal macrophages was measured using a

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migration assay in the presence and absence of PF4178903. Original magnification is x200 (scale bar = 100 μm).*P < 0.05 compared to control. #P < 0.05 compared to CCL2, CCL4 or ATCM treated

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3. PF4178903 attenuates weight gain in HFD-fed mice

To examine whether PF4178903 influenced diet-induced obesity, we investigated body weight in mice fed either a RD or HFD, both with and without PF4178903 (10 mg/kg/day), for 12 weeks. The HFD-fed mice had significantly increased body weights compared to the RD-fed mice. Body weights did not differ between the RD-fed control mice and PF4178903 treated RD-fed mice. However, the HFD-fed mice treated with PF4178903 had significantly attenuated body weight gains compared to HFD-fed control mice (Figure 3a), whereas food intake was similar among the two groups (Figure 3b).

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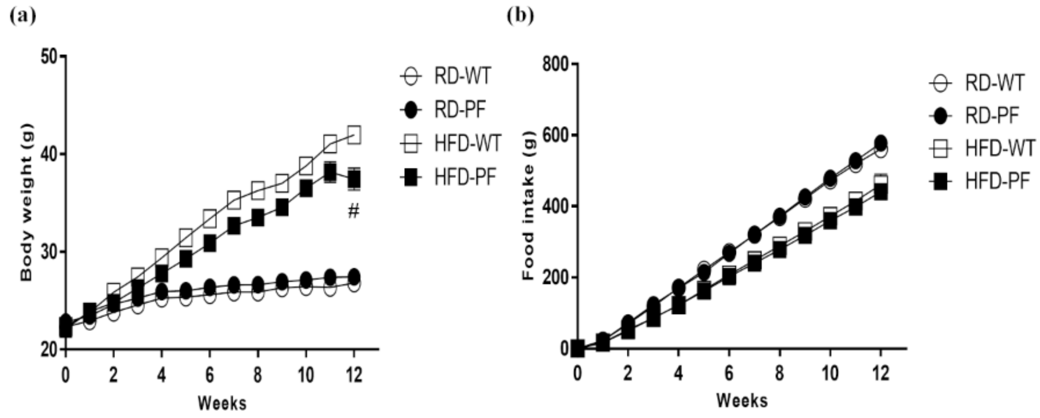

Figure 3. PF4178903 attenuates weight gain in HFD-fed mice. WT and PF4178903 treated mice were fed HFD or RD for 12 weeks. (a) Body weight changes over time (b) Food intake changes over time. (n=10 for each group; #P < 0.05 compared to HFD-fed WT mice).

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4. PF4178903 reduces adipocyte size in HFD-fed mice

We further examined the histological analyses of fixed epididymal adipose tissue and quantified adipocyte size. Hematoxylin and eosin staining of adipose tissue showed significant expansion in the size of adipocytes in epididymal fat of HFD-fed mice. In addition, HFD-fed mice showed crown-like structures (CLS) which represent an accumulation of macrophages around dead adipocytes. However, PF4178903 treatment significantly attenuated these effects (Figure 4a-b). The weight of epididymal fat mass was not significantly different between HFD mice with PF4178903 treatment and HFD-fed control mice (Figure 4c). Analyses of the adipocyte size distribution in epididymal adipose tissue revealed that HFD-fed mice had a significant shift toward larger adipocytes, whereas in HFD-fed mice treated with PF4178903 this shift was prevented and a normal distribution profile of adipocyte size was restored (Figure 4d).

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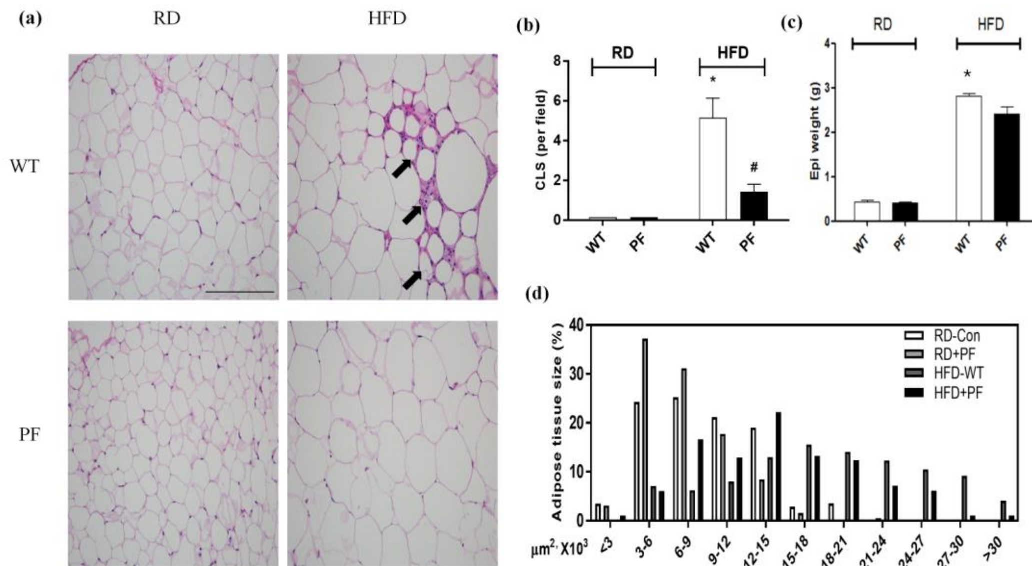

Figure 4. PF4178903 reduces macrophage infiltration and adipocyte size in adipose tissue of HFD-fed mice. (a) Hematoxylin-eosin stained section of epididymal adipose tissue. Representative images are shown. Scale bar, 200 µm. (arrow = crown like structures) (b) Number of crown-like structures (CLS) in epididymal adipose tissue (c) Weight of epididymal adipose tissue. (d) Adipocytes size distribution of epididymal adipose tissue. (Data are shown as mean ± SEM with n = 10 animals per group. *P <0.05 compared with the RD-fed mice. #P < 0.05 compared to HFD-fed WT mice).

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5. PF4178903 attenuates HFD-induced metabolic dysfunction and inflammation

To investigate the effects of PF4178903 on biochemical parameters, we measured metabolic parameters in mice. PF4178903 treatment attenuated the HFD-induced higher levels of serum glucose and insulin seen in HFD control mice. Serum levels of total cholesterol, triglyceride and HOMA-IR values were significantly lower in PF4178903 treated HFD-fed mice compared with HFD control mice. Serum adiponectin levels were higher in HFD-fed mice treated with PF4178903 compared to levels measured in HFD-fed control mice (Table 1). Systemic inflammation status was also evaluated, and the results showed that PF4178903 treatment markedly reduced serum levels of pro-inflammatory cytokines, including TNF-α, MCP-1 and IL-6, in HFD-fed mice (Figure 5a, b, c). These data indicated that PF4178903 treatment inhibited HFD-induced inflammation.

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Table 1. Effect of PF4178903 on metabolic parameters at 12 weeks of treatment

	RD	RD+ PF4178903	HFD	HFD+ PF4178903
Serum glucose (mg/dl)	139.23±7.51	130.78±4.80	187.67±5.90*	151.80±8.97#
Serum insulin (ng/dl)	0.20±0.01	0.19±0.03	0.39±0.05*	0.23±0.01#
Serum total cholesterol (mg/dl)	155.79±3.92	154.57±5.29	266.13±3.36*	227.13±6.57#
Serum triglyceride (mg/dl)	34.21±2.70	27.76±2.66	70.97±3.66*	45.14±2.39 #
HOMA-IR	0.03±0.00	0.02±0.00	0.07±0.00*	0.03±0.00#
Serum adiponectin (μg/ml)	32.32±0.89	29.07±0.96	19.87±0.37*	23.18±0.55#

RD, regular diet; HFD, high fat diet; HOMA-IR, homeostatic model assessment of insulin resistance

Data are means ± SEM and *$P < 0.05$ vs. compared to RD fed mice

#$P < 0.05$ compared to HFD fed mice.

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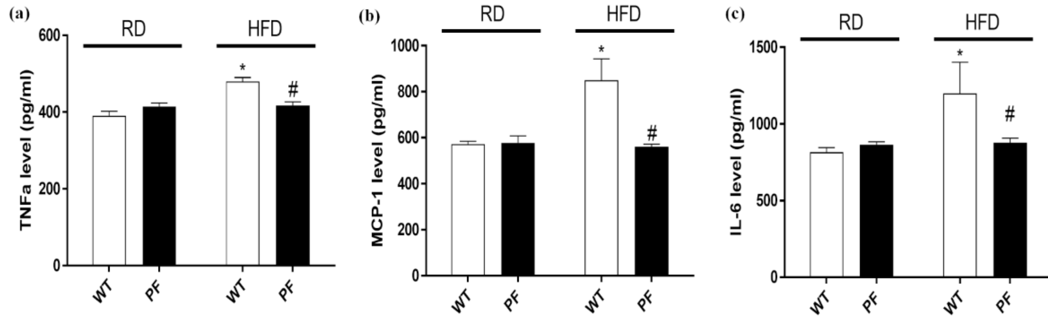

Figure 5. PF4178903 attenuates HFD-induced inflammation. Protein levels of cytokines in the serum were determined by flow cyometry analysis. (a) TNF-α, (b) MCP-1 and (c) IL-6. (Data are shown as mean ± SEM with n = 10 animals per group. *P <0.05 compared with the RD-fed WT mice. #P <0.05 compared with the HFD-fed WT mice.)

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6. PF4178903 attenuates HFD-induced impaired glucose tolerance and improves insulin sensitivity in adipose tissue

The result of intraperitoneal glucose tolerance test revealed that plasma glucose levels were significantly decreased in a time-dependent manner in PF4178903 treated HFD-fed mice compared to HFD-fed control mice (Figure 6a). Similarly, the result of insulin tolerance test revealed that PF4178903 treated HFD-fed mice displayed increased insulin sensitivity when compared with HFD control mice (Figure 6b). Furthermore, PF4178903 treatment increased the levels of phosphorylated IRS-1 and phosphorylated AKT in white adipose tissue, which were reduced by the high fat diet (Figure 6c). These data suggest that PF4178903 prevents HFD-induced insulin resistance.

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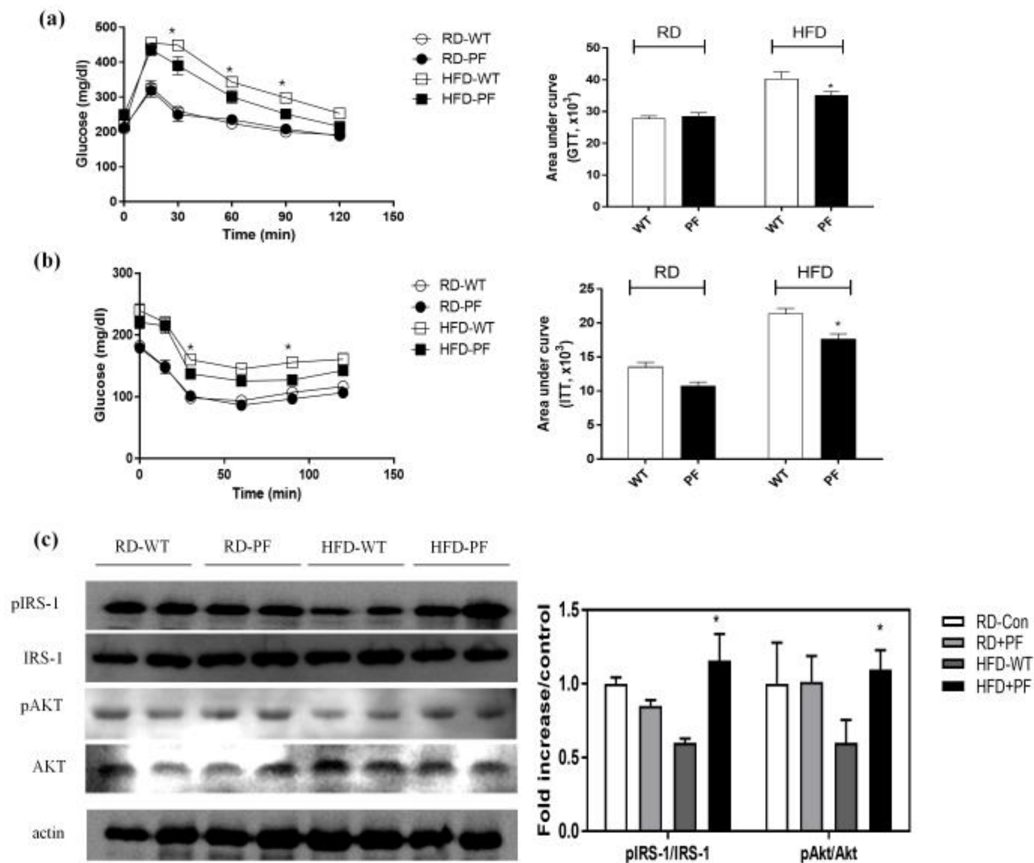

Figure 6. PF4178903 attenuates HFD-induced impaired glucose tolerance and improves insulin sensitivity in adipose tissue. (a) Mice underwent glucose (1 g/kg) tolerance tests and area under the curve of glucose tolerance test (GTT). (b) Insulin (0.75 U/kg) tolerance tests (ITT) and area under the curve of ITT. (c) Western blots of insulin signaling molecules, pIRS-1 and pAkt in the adipose tissue. Cropped membrane was used in Western blot. *P < 0.05 compared to HFD-fed WT mice.

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7. PF4178903 regulates M1/M2 status and reduces CD8 T cell numbers in epididymal adipose tissue

We next examined the phenotype of immune cell after PF4178903 treatment in adipose tissue using flow cytometric analyses. The increased total numbers of ATMs associated with HF feeding were significantly decreased by PF4178903 treatment (Figure 7b). We used antibodies against CD11c and CD206 to discriminate M1 and M2 macrophages, respectively. The increased percentages of CD11c-positive M1 ATMs in HFD mice were reduced after PF4178903 treatment while, in contrast, the percentage of CD206-positive M2 ATMs was well preserved in HFD-fed mice treated with PF4178903 (Figure 7a,c and d). In addition, whereas the percentage of CD8+ T cells was higher in HFD-fed mice, PF4178903 treatment significantly reduced the percentage of CD8+ T cells in HFD-fed mice. However, PF4178903 treatment did not significantly lower the percentage of CD4+ T cells in HFD-fed mice (Figure 7e-g).

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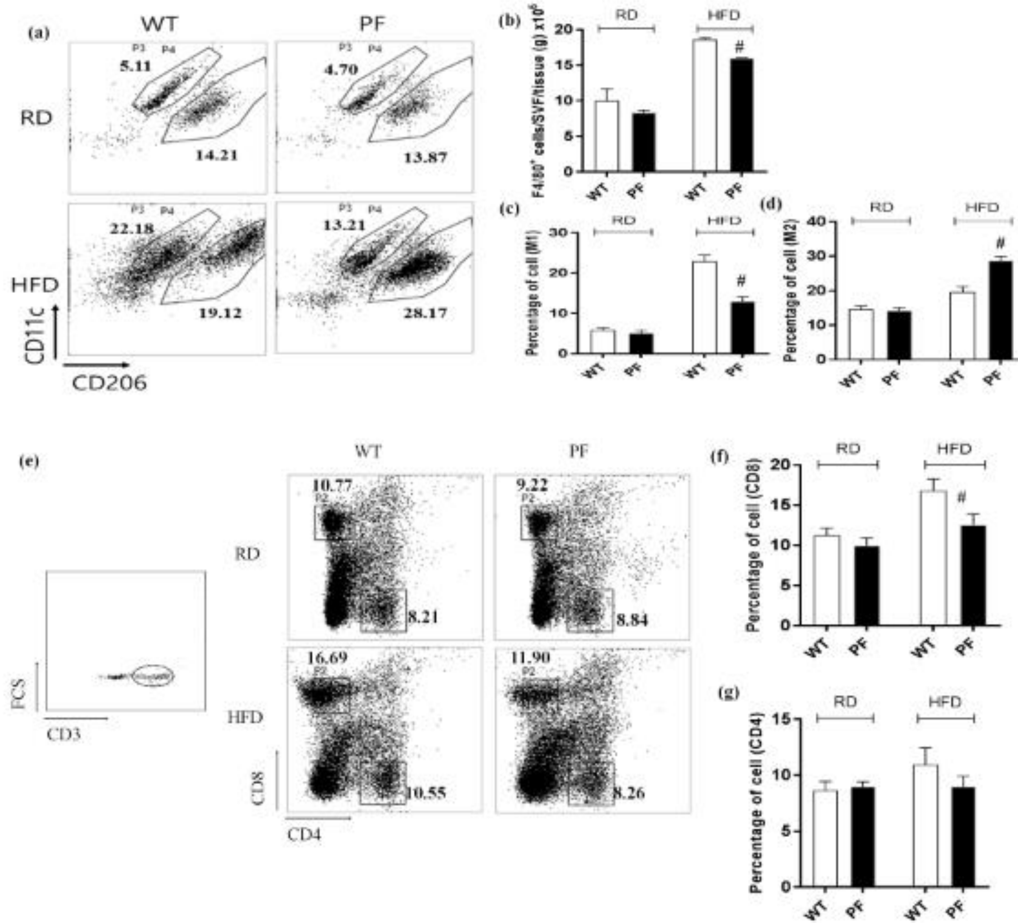

Figure 7. PF4178903 decreased M1-type and increased M2-type macrophages in WAT of HFD-fed mice. Flow cytometry analysis of the stromal vascular fraction (SVF) from epididymal adipose tissue of mice. (a) Representative flow cytometric plots of the M1 macrophages represented by the F4/80+ CD11c+ and M2 macrophages represented by the F4/80^{+} CD206^{+} (b) Quantification of total adipose tissue macrophages (c) Percentages of M1 adipose tissue macrophages (d)

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Percentages of M2 adipose tissue macrophages (e) FACS plots and gating of the stromal-vascular cells to detect adipose tissue T cells (CD3+ cells). Representative flow cytometric plots of the CD8+ T cell (CD3+/CD4-/CD8+) and CD4+ T cell (CD3+/CD4+/CD8-) (f) Percentages of CD8^{+} T cells, (g) Percentages of CD4^{+} T cells. $^{\#}P < 0.05$ compared with the HFD-fed WT mice.

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IV. DISCUSSION

Our study provides firm evidence of a crucial role for CCR2 and CCR5 in regulating the macrophage-mediated inflammatory response to obesity and the development of insulin resistance. We demonstrated that the administration of a dual CCR2/CCR5 antagonist, PF4178903, attenuated HFD- induced metabolic dysfunction, as demonstrated by reduced body weight gain, lower plasma glucose and lipids levels, reduced adipocyte size and systemic inflammation, as well as improved glucose tolerance and insulin sensitivity. The mechanisms of which were associated with regulating the balance of M1/M2 macrophage polarization in adipose tissue. Moreover, using in vitro experiments, we also confirmed that PF4178903 abrogated chemokine-induced macrophage migration.

Chemokine receptors have become attractive targets for anti-obesity therapy, as interactions with their ligands mediate the recruitment of inflammatory cells to the adipose tissue, and contribute to chronic inflammation and insulin resistance.[13] Although clinical trials had been conducted to determine the effect of a CCR2 inhibitor in human metabolic diseases, these inhibitors were not developed as therapeutic agents.[14] This suggests that other chemokine receptors may also play significant roles in insulin resistance and chronic inflammation.

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There is increasing evidence that CCR5 also plays a considerable role in the regulation of the adipose tissue inflammatory response to obesity.[9] As in a previous report, our results demonstrated that both CCR2 and CCR5 are expressed in adipose tissue macrophages of diet induced-obesity mice (Figure 1). In this background, novel agents which simultaneously antagonize multiple chemokine receptors, such as dual CCR2 /CCR5 antagonists have been developed.[15, 16] However, to date, there have been few studies investigating the effect of a dual chemokine receptor antagonist on obesity related chronic metabolic disease. Therefore, we focused this study on the role of dual CCR2 /CCR5 antagonists in obesity and insulin resistance.

Our results showed that PF4178903 significantly improved HFD-induced metabolic dysfunction, insulin resistance and inflammation via a reduction of ATMs infiltration. Adipose tissue is an important endocrine organ. In addition to its role in storing energy, HFD-induced obesity primes inflammation in adipose tissue, suggesting that a dysfunction of adipose tissue strongly contributes to the initiation and exacerbation of insulin resistance.[17] With respect to inflammation in adipose tissue, ATMs recruitment is a critical factor in the pathogenesis of insulin resistance.[18] We observed that PF4178903 reduced crown-like structures, an indicator of the extent of macrophage recruitment and the number of dead

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adipocytes,[19] in the adipose tissue of HFD-fed mice. Subsequently, PF4178903 treatment reduced chronic systemic inflammation and improved insulin sensitivity via regulating ATMs recruitment in adipose tissue.

In the present study, PF4178903 significantly attenuated obesity-induced body weight gain, while it did not affect food intake. Furthermore, we observed that PF4178903 suppressed adipocyte hypertrophy in epididymal white adipose tissue, consequently increasing serum adiponectin levels in HFD-fed mice. Oversized adipocytes release free fatty acid (FFAs) by failing to store and oversized adipocytes also release chemokines (e.g.MCP-1, CCL4).[20] The excessive release of FFAs and chemokines affects ATMs signaling by promoting pro-inflammatory activation, and a vicious circle evolves between hypertrophied adipocytes and ATMs.[21] Our in vitro result demonstrated that PF4178903 markedly inhibited chemokine induced-macrophage chemotaxis. These findings contribute to the growing evidence for cross-talk between adipocytes and macrophages in modulating immune and metabolic functions.

Dysregulation of M1/M2 polarization in macrophages as well as macrophage recruitment has become an emerging mechanism underlying the pathogenesis of obesity and insulin resistance.[22] Thus, strategies restraining M1 polarization and/or driving alternative M2 activation of macrophages may have

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the potential to protect against exacerbated inflammation and insulin resistance in obesity. Here, we showed that PF4178903 significantly shifted the macrophage phenotype from M1 (CD11c+) to M2 (CD206+) in HFD-induced obesity. These data imply that the dual CCR2/CCR5 blockade regulates macrophage polarization in ATMs. Although the exact mechanism of shifting macrophage phenotype by dual CCR2/CCR5 blockade can not be explained in this present study, we assume that reversed adipocyte hyperplasia and lowered adipocyte death by PF4178903 treatment causes M2 shift of macrophage.[19, 23] In addition, increased secretion of adiponectin from relatively lean phenotype adipocyte caused by PF4178903 treatment might contribute to M2 shift of macrophage.[24]

Recent reports have shown that the infiltration of CD8+ T cells precedes M1-polarized macrophage recruitment, and the interactions between T cells and macrophages are considered to constitute a maladaptive feed-forward loop, which leads to adipose inflammation and insulin resistance.[25, 26] We also observed that PF4178903 specifically reduced the accumulation of CD8+ T cells but did not influence CD4+ T cells in adipose tissue (Figure 7d-f). This finding supports the fact that changes in the immune system might precede macrophage polarization, and that ATMs might be effectors of a coordinated inflammatory response.[27] These adaptive immune responses induced by PF4178903 in obesity might be

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beneficial and function to preserve metabolic homeostasis.

V. CONCLUSION

Collectively, our results indicate that blocking both CCR2 and CCR5 maintains metabolic and immune homeostasis via regulating adipose tissue macrophages recruitment and polarization toward an anti-inflammatory phenotype, thereby attenuating obesity-induced inflammation and insulin resistance. Overall, the current investigation highlights the potential clinical utility of dual CCR2/CCR5 antagonist in the attenuation of macrophage-mediated inflammation and the prevention of obesity-induced insulin resistance will provide a therapeutic strategy for combating obesity related metabolic diseases.

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국문요약

케모카인수용체 2/5 동시 저해제가 대식세포의 집합과 M1/M2 상태를 조절함으로써 비만에 의한 인슐린 저항성을 개선시킨다

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의학과

케모카인수용체 2와 케모카인수용체 5를 통한 대식세포의 집합에 의해 유도되는 지방조직내 만성 염증은 비만 관련 만성 질환과 인슐린 저항성에 있어 중요한 병인으로 작용한다. 하지만, 아직까지 이 두가지 수용체를 타깃으로 비만에서의 효과를 보고자 했던 연구는 없었다. 따라서, 본 연구에서는 케모카인수용체 2와 5를 동시에 억제시킬 수 있는 PF4178903을 이용하여 케모카인수용체 2와 5를 동시 억제가 비만과 인슐린 저항성에 미치는 영향을 보고자 하였다. 본 연구에서는 40마리 8주령의 수컷 C57BL/6J mice를 다음과 같이 식이와

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약물 처리에 따라 4가지 그룹으로 나누었다. 있다: (1) 정상 식이 군, (2) 정상식이를 하면서 PF4178903을 경구로 주입한 군 (3) 고지방식이군 (4) 고지방식이를 하면서 PF4178903을 경구로 주입한 군. 이들은 총 12주간 해당 식이와 약제 투여를 유지하고, 이후, 부검하였다. 그 결과, PF4178903을 투여한 군에서는 고지방식이를 하더라도 체중증가가 적고, 지방조직을 구성하는 비만세포의 크기도 작으면서 지방조직 내에 대식세포의 숫자가 감소하는 것을 확인할 수 있었다. 또한 PF4178903로 인해 혈중에서 염증성 시토카인과 혈당, 그리고 중성지방 및 콜레스테롤 수치가 감소하며, 인슐린 저항성이 개선되는 효과를 보였다. 그리고, 지방 조직 내 면역세포의 구성 변화를 확인하기 위해 FACS 분석을 하였는데, PF4178903을 투여함으로써 대식세포의 유형이 M1에서 M2로 변화하고, CD8 T 세포의 숫자가 감소하는 것을 확인할 수 있었다. 따라서, 케모카인수용체 2/5를 동시억제는 지방 조직 내에 면역세포의 구성의 변화를 통해 비만에서 이루어지는 염증과정이 완화시킨다. 이러한 결과를 바탕으로 케모카인수용체 2/5 동시저해제의 비만 관련 만성질환의 예방 또는 치료적 목적의 활용에 대해 기대해볼

수 있겠다.

핵심되는 말: 케모카인, 비만, 인슐린저항성, 염증, 대식세포