DPP-IV inhibitor anagliptin exerts anti-inflammatory effects on macrophages, adipocytes, and mouse livers by suppressing NF-κB activation

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This study aimed to investigate both the in vitro and the in vivo effects of a DPP-IV inhibitor, anagliptin, on inflammation. First, the effects of anagliptin on cytokine productions and nuclear factor-κB (NF-κB) transcriptional activity were determined using lipopolysaccharide (LPS)- or palmitate-stimulated RAW264.7 macrophages. Then, 3T3-L1 adipocytes were cocultured with RAW264.7 macrophages, and the effects of anagliptin on these adipocytes were examined in the presence or absence of LPS stimulation. Finally, using LPS-infused mice, we examined the effects of anagliptin on hepatic and adipose cytokine productions, serum cytokine concentrations, and hepatic NF-κB transcriptional activity. We herein present evidence suggesting that anagliptin improves the inflammation affecting adipose tissue and hereby exacerbating macrophage infiltration, thereby improving insulin resistance. This vicious cycle involving macrophages and adipocytes contributes to the pathogenesis of chronic inflammation and insulin resistance (25, 31, 45).

Anagliptin also acted on 3T3-L1 adipocytes, weakly suppressing NF-κB transcriptional activity in LPS-infused mice. Taking these observations together, the anti-inflammatory properties of anagliptin may be beneficial in terms of preventing exacerbation of diabetes and cardiovascular events.

CHRONIC INFLAMMATION IS ONE OF THE CAUSES OF insulin resist-

ANCE in type 2 diabetes, which is associated with a high rate of obesity as a background factor (21, 32, 33). In the obese state, macrophages infiltrate adipose tissues and interact with enlarged adipocytes, thereby exacerbating adipocyte inflammation and insulin resistance (8, 15, 18, 40, 43). This vicious cycle involving macrophages and adipocytes contributes to the pathogenesis of chronic insulin resistance (12, 17, 44, 48). To date, whereas numerous investigations have focused on the effects of DPP-IV inhibitors on insulin and/or glucagon secretion, less attention has been paid to the impacts of these drugs on insulin resistance or inflammation. Some reports have shown GLP-1 to exert anti-inflammatory effects (6, 22, 26), whereas others found no evidence of DPP-IV inhibitor agents directly improving chronic inflammatory states.

Dipeptidyl peptidase-IV (DPP-IV) inhibitors were developed to enhance glucose-induced insulin secretion and prolong the activities of incretins such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Importantly, DPP-IV expression is reportedly upregulated in chronic inflammatory diseases such as atherosclerosis, multiple sclerosis, inflammatory bowel disease, and obesity, suggesting DPP-IV involvement in the pathogenesis of inflammation (16, 17, 35, 44, 48). To date, whereas numerous investigations have focused on the effects of DPP-IV inhibitors on insulin secretion, less attention has been paid to the impacts of these drugs on insulin resistance or inflammation.
METHODS

Materials. Anagliptin was provided by Sanwa Kagaku Kenkyusho. The antibodies against inhibitor-xBox (IxBx), p65, phospho-p65, c-Jun NH2 terminal kinase (JNK), phospho-JNK, p38, and phospho-p38 were purchased from Cell Signaling Technology (Beverly, MA). Anti-actin antibody and cycloheximide were purchased from Sigma-Aldrich (St. Louis, MO). Anti-rabbit and anti-mouse horseradish peroxidase-conjugated antibodies were obtained from GE Healthcare (Buckinghamshire, UK). All other reagents were of analytical grade.

Animals. BALB/c mice were housed under climate-controlled conditions with a 12:12-h light-dark cycle and were provided standard food and water ad libitum. All protocols were approved by the Institutional Review Board of Hiroshima University.

Cells and cell culture. Mouse 3T3-L1 preadipocytes [American Type Culture Collection (ATCC), Manassas, VA] were maintained in Dulbecco’s modified Eagle’s medium (DMEM; Nissui Pharmaceutical, Tokyo, Japan) containing 10% donor calf serum in an atmosphere of 10% CO2 at 37°C. Two days after 3T3-L1 fibroblasts had reached confluence, differentiation was induced by treating the cells with DMEM containing 4 µg/ml dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 200 nM insulin (Cell Science & Technology Institute, Sendai, Japan), and 10% fetal bovine serum (FBS; Biowest) for 48 h, as described previously (1, 2). Cells were fed DMEM supplemented with 10% FBS every other day and used as mature 3T3-L1 adipocytes on day 8 after the induction of differentiation unless otherwise noted.

The murine macrophage cell line RAW264.7 was obtained from ATCC. RAW264.7 and 3T3-L1 were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin G, and 100 µg/ml streptomycin. Coculture of adipocytes and macrophages was performed using a transwell system (Corning, Acton, MA) with a 0.4-µm porous membrane to separate the upper and lower chambers. One × 106 differentiated 3T3-L1 adipocytes were cultured in the lower chamber, whereas 5 × 105 RAW macrophages were cultured in the upper chamber (46).

The cells were treated with the indicated concentrations of Escherichia coli LPS (Sigma), palmitate, or tumor necrosis factor-α (TNFα) in the presence or absence of angliptin or sitagliptin for the indicated times.

Measurement of DPP-IV activities in RAW cells, 3T3-L1 cells, medium, and tissues. After RAW264.7 cells (2 × 106/cells) had been incubated with or without 10 ng/ml LPS for 24 h, the cells were pelleted and the culture medium was harvested. 3T3-L1 cells were also pelleted, and culture media were harvested on days 0 and 8. For isolation of peritoneal macrophages from C57BL/6J mice, the mice were injected intraperitoneally with 1 ml of 3% thioglycollate medium (Becton-Dickinson, Tokyo, Japan). Four days later, the elicited cells were collected from the peritoneal cavity by lavage with 5 ml of ice-cold phosphate-buffered saline (PBS) twice. The cells were plated onto six-well plates in DMEM (Sigma) supplemented with 10% FBS and then cultured at 37°C for 1 h to achieve adherence of the cells to the plates. Nonadherent cells were subsequently removed by washing with PBS. Pelletized RAW and 3T3-L1 cells and the adherent cells were lysed by sonication with extract buffer (25 mM HEPES, 140 mM NaCl, 1% Triton X-100, and 30 IU aprotinin, pH 7.8). The kidneys, liver, pancreas, and thoracic aorta were collected from C57BL/6J mice and lysed employing extract buffer. Each lysate was then centrifuged at 14,000 rpm for 10 min. Glycerol was added to the supernatant to achieve a final concentration of 20% for determination of DPP-IV activity in the cell lysate. All of the aforementioned methods were performed on ice. Each sample was reacted with 0.18 mg/ml bovine serum albumin-containing HEPES buffer, with glycyl-l-proline 4-methylcoumarin-7-amide (Gly-Pro-MCA; final concentration: 0.18 mM/l; Peptide Institute, Osaka, Japan) added. DPP-IV activities were calculated based on the cleavage rate of 7-amino-4-methylcoumarin (AMC) from Gly-Pro-MCA in response to DPP-IV, using AMC standard curves, as described previously (23, 24).

To examine the DPP-IV inhibitory rates of angliptin and sitagliptin, 10 or 100 µM angliptin or sitagliptin was added to the aforementioned RAW264.7 and 3T3-L1 cell samples and culture medium, followed by incubation for 30 min at room temperature. Then, DPP-IV activities were measured. DPP-IV activity was expressed as the amount of cleaved AMC per minute per milligram of protein and per milliliter.

Western blot analysis. The cells were solubilized with Laemmli buffer (0.2 M Tris·HCl, 4% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.1% bromophenol blue). Equal amounts of protein from whole cell lysates were resolved by SDS-PAGE. The proteins were then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA) using an electroblotting apparatus (Mighty Small Transphor; GE Healthcare, Waukesha, WI) and subjected to immunoblotting using the Super Signal West Pico Chemiluminescence System (Thermo Fisher Scientific, Rockford, IL). The results of several immunoblots were quantitatively analyzed using Chemi Doc XR Plus and Image Lab SVP software (Bio-Rad Laboratories, Hercules, CA).

Transfection and luciferase reporter assay. One day before transfection, the cells were plated to achieve 80–90% confluence. RAW264.7 cells were transfected with NF-κB luciferase reporter, activation protein-1 (AP-1) luciferase transplasmid (Clontech, Palo Alto, CA), and pRL-TK Vector (Promega, Madison, WI) to normalize transfection efficiency, using X-tremegene 9 DNA Transfection Reagent (Roche Applied Science, Basel, Switzerland) according to the manufacturer’s instructions. The cells were left overnight and then incubated with or without 10 ng/ml LPS or 100 µM angliptin for an additional 2 and 12 h. The cells were then washed with PBS, and luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega).

Measurement of mRNA expression by real-time PCR. RAW264.7 or 3T3-L1 cells were treated with 10 ng/ml LPS, TNFα, or palmitate in the presence or absence of angliptin or sitagliptin for the indicated times. Total RNA was isolated using Sepazol-RNA 1 (Nakalai Tesque, Kyoto, Japan), and 1 µg of RNA was reverse transcribed with Transcripter Reverse Transcriptase (Roche). The amplification reaction was performed using SYBR Premix Ex Taq (TaKaRa, Shiga, Japan) according to the manufacturer’s protocol. The primers were as follows: mouse IL-1β: forward 5'-CTCGCTACGGGTCTCAAGAAAC-3', mouse IL-1β reverse 5'-CATCAGAGGCCAGAGAGAAGC-3'; mouse TNFα forward 5'-GCCACACGCCTTCTCTGCT-3', mouse TNFα reverse 5'-GCTGCGCCATAGAACTGAT-3'; mouse IL-6 forward 5'-GATGCTTTACAACTGTTATATC-3', mouse IL-6 reverse 5'-GATGTCCACTGGCtgttcttGTC-3'; mouse IL-12 reverse 5'-CTTCTGCGCCAGAAACCTC-3'; mouse IL-12 reverse 5'-CACCTTTGTCAGTTGTCACAGC-3'; mouse monocyte chemoattractant protein-1 (MCP-1) forward 5'-CTTTGTCGCTGCTGTGTC-3', mouse MCP-1 reverse 5'-CTTTGCGCTGGCCTGATAA-3', mouse GAPDH forward 5'-AGGTTCCTTCAACCACCT-3'; mouse GAPDH reverse 5'-GGACTGACCCAGTTGACTGAC-3'. Post-PCR melting curves confirmed the specificity of single-target amplification. Fold changes in the expressions of TNFα, IL-1β, IL-6, IL-12, and MCP-1 relative to GAPDH were determined in triplicate.

ELISA. Releases of TNFα, IL-6, and MCP-1 in the supernatants of RAW264.7 macrophages and cocultured 3T3-L1 adipocytes, each treated with 10 ng/ml LPS in the presence or absence of angliptin, were measured using ELISA kits (eBioscience, San Diego, CA) according to the manufacturer’s instructions. Absorbance at 450 nm was determined using a microplate reader (Bio-Rad). Similarly, the serum concentrations of TNFα, IL-6, and MCP-1 in mice with or without LPS injection were measured employing the same assay kits.

Adenoviruses. Adenovirus containing the NF-κB response element and luciferase cDNA (Ad-NF-κB-Cre) was generated using an adenovirus dual expression kit (TaKaRa) according to the manufacturer’s instructions.
Anagliptin suppresses inflammatory cytokine mRNA expressions on LPS-stimulated macrophages. RAW264.7 macrophages were stimulated with or without 10 ng/ml LPS for 2, 6, 12, and 24 h, and the effects of 10 or 100 μM anagliptin and sitagliptin were examined. Anagliptin significantly and dose-dependently suppressed LPS-induced increases in TNFα, IL-1β, IL-6, and IL-12 mRNA expressions in LPS-stimulated RAW264.7 macrophages (Fig. 1A). The other DPP-IV inhibitor sitagliptin also exerted an anti-inflammatory effect, but this effect appeared to be weaker than that of anagliptin (Fig. 1B). Similar effects of anagliptin were also observed when the cells were stimulated with 200 μM palmitate for 2 h (Fig. 1C). Additionally, anagliptin also significantly inhibited palmitate-induced mRNA expression of TNFα, IL-6, and MCP-1 in 3T3-L1 adipocytes (Fig. 1D). These results indicate that DPP-IV inhibitors, both anagliptin and sitagliptin, exert anti-inflammatory effects on RAW264.7 cells.
DPP-IV activity in RAW264.7 macrophages was low but further suppressed by anagliptin or sitagliptin. DPP-IV protein in RAW264.7 macrophages was undetectable by immunoblotting, suggesting a low expression level. To confirm the existence of DPP-IV expression in RAW264.7 cells and examine whether the higher suppressive effect of anagliptin than of sitagliptin on cytokine mRNA expressions might be attributable to a difference in the inhibitory activities of these two drugs, DPP-IV activities were measured in RAW264.7 macrophages and in culture medium (Fig. 2, A and B). Among the mouse tissues examined, the liver and kidneys had the highest DPP-IV activity, whereas the levels were low in the pancreas and thoracic aorta. The DPP-IV activity of RAW264.7 cells was revealed to be even lower than that in mouse primary macrophages while being unaffected by LPS stimulation. Additionally, RAW264.7 cells and undifferentiated (day 0) and differentiated (day 8) 3T3-L1 cells showed similar DPP MCP-1 activities (Fig. 2A). DPP-IV activity in the medium was unaffected by LPS stimulation of RAW264.7 cells or by adipose differentiation of 3T3-L1 cells (Fig. 2B).

The inhibitory effects of 100 μM anagliptin and sitagliptin on DPP-IV activity in cell lysates were approximately double that of the 10 μM respective doses, but the DPP-IV activity in the supernatant was strongly inhibited by a concentration of even 10 μM of anagliptin or sitagliptin (Fig. 2C). The respective DPP-IV activities in the RAW264.7 and 3T3-L1 cells incubated with each concentration of Ana and Sita for 30 min were determined to be concentration dependent. D: DPP-IV activities in RAW and 3T3-L1 cells incubated with each concentration of Ana and Sita for 30 min were determined to be concentration dependent.

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Fig. 2. Measurements of dipeptidyl peptidase-IV (DPP-IV) activity in mouse tissues, peritoneal macrophages, and cell lysates and conditioned medium of RAW264.7 (RAW) and 3T3-L1 cells. RAW cells were stimulated with or without LPS for 24 h. DPP-IV activity in mouse kidney, liver, pancreas, thoracic aorta, peritoneal macrophages, and RAW and 3T3-L1 cell lysates (A) and conditioned medium (B) were determined. C: DPP-IV inhibitory effects of 10 or 100 μM Ana and Sita on RAW and 3T3-L1 cell lysates and supernatants were determined to be time dependent. D: DPP-IV activities in RAW and 3T3-L1 cells incubated with each concentration of Ana and Sita for 30 min were determined to be dose dependent.
with 100 μM sitagliptin treatment (Fig. 2D). This data series indicates that DPP-IV activities in RAW264.7 and 3T3-L1 cells, although not high, are readily measurable and are suppressed by DPP-IV inhibitors.

**Anagliptin inhibits LPS-induced NF-κB activation via p65 phosphorylation and the phosphorylations of JNK, p38, and AP-1 activity in RAW264.7 macrophages.** To elucidate the molecular mechanism underlying anagliptin-induced suppression of inflammatory cytokines, we investigated the signal transduction to NF-κB and AP-1 activations in LPS-stimulated RAW264.7 macrophages. Stimulation with LPS induced IκBα degradation and phosphorylations of JNK and p38 (Fig. 3A). Coincubation with anagliptin significantly, albeit not markedly, suppressed LPS-induced phosphorylations of JNK1 and p38 that had been initiated mainly 25–30 min after the stimulations (Fig. 3, A and B). The complex of p50 and phosphorylated p65 is reportedly involved in NF-κB activation, and phosphorylation of p65 was also revealed to be significantly suppressed by anagliptin during the period from 30 to 120 min after the stimulation (Fig. 3, C and D). Next, NF-κB and AP-1 transcriptional activities were examined using the luciferase assay. As shown Fig. 3E, whereas LPS stimulation enhanced the transcriptional activities of NF-κB by approximately six- and 15-fold and of AP-1 by ~1.5- and 4.5-fold at 2 and 12 h, respectively, anagliptin partially but significantly suppressed both increases, which appears to be reflected by the suppressions of p65 phosphorylation and of JNK and p38/AP-1 activities.

**Anagliptin attenuated inflammatory cytokine expressions in 3T3-L1 adipocytes treated with LPS and TNFα or cocultured with LPS-stimulated RAW264.7 macrophages.** First, we examined whether or not anagliptin and sitagliptin act directly on 3T3-L1 adipocytes stimulated with LPS. LPS stimulation clearly induced expressions of IL-6 and MCP-1, but not of TNFα or IL-12, when applied during the period from 2 to 24 h (Fig. 4, A and B). Anagliptin partially but significantly inhibited LPS-induced IL-6 and MCP-1 mRNA expressions in a dose-dependent manner (Fig. 4A). Sitagliptin exerted the same effect, albeit more weakly than anagliptin (Fig. 4B). Second, we investigated whether or not each of these two
DPP-IV inhibitors acts on 3T3-L1 adipocytes stimulated with TNFα. TNFα markedly induced mRNA expressions of TNFα, IL-6, IL-12, and MCP-1 (Fig. 4, C and D). Anagliptin partially but significantly reduced the mRNA levels of TNFα, IL-6, IL-12, and MCP-1, which had been elevated by TNFα stimulation, in a dose-dependent manner (Fig. 4C). The same, albeit slightly weaker, trend was observed in the presence of sitagliptin (Fig. 4D). Then, 3T3-L1 adipocytes were cocultured with RAW264.7 macrophages using a transwell system, and the effect of anagliptin on the LPS-induced response to adipocytes was examined. Anagliptin was demonstrated to significantly inhibit mRNA expressions of inflammatory adipokines such as TNFα, IL-6, IL-12, and MCP-1 (Fig. 5A). The concentrations of TNFα, IL-6, and MCP-1 in the medium were also reduced in response to the addition of anagliptin (Fig. 5B). In these experiments, the effect of anagliptin was significant at the 10 μM concentration, but it was even more marked at 100 μM (Fig. 5, A and B).

Anagliptin attenuates LPS-induced NF-κB activation in the mouse liver. To investigate whether anagliptin also exerts an inhibitory effect on LPS-induced NF-κB activation in vivo, we measured the LPS-stimulated NF-κB activity in mouse livers. C57BL/6J mice that had been infected with Ad-NF-κB-Cre by tail vein injection were divided into four groups: PBS, 10 mg/kg anagliptin, 5 mg/kg LPS, and 5 mg/kg LPS with 10 mg/kg anagliptin. Whereas mice intraperitoneally injected with LPS showed strong enhancement of NF-κB activity, anagliptin significantly attenuated NF-κB activation slightly but significantly at 2 h and markedly at 6 and 12 h after LPS infusion (Fig. 6, A and B). At 2 h hepatic NF-κB activity was low, and this was attributed to luciferase protein production possibly not being sufficient for fluorescent reaction, as described in a previous report (5). These results suggest the inhibitory effect of anagliptin on NF-κB activation and possibly its resultant inflammatory response to be present not only in vitro but also in mouse tissues.

Anagliptin attenuates LPS-induced elevations of hepatic and adipose mRNA levels and serum concentrations of inflammatory cytokines. In good agreement with the NF-κB activation data, LPS-induced elevations of MCP-1, IL-1β, IL-6, and IL-12 were significantly attenuated by the administration of anagliptin, although the TNFα reduction did not reach statistically significant differences compared with the LPS group.
tactical significance (Fig. 6C). Similar results were obtained for adipose tissues, in which LPS-induced elevations of TNFα, MCP-1, IL-1β, IL-6, and IL-12 were all significantly suppressed by the administration of anagliptin (Fig. 6D). Furthermore, serum concentrations of TNFα, IL-6, and MCP-1 were also markedly elevated in response to LPS infusion, but these elevations were reduced to one-half by the administration of anagliptin (Fig. 6E). On the other hand, the serum active GLP-1 level was elevated by anagliptin and showed no change in response to administration of LPS (Fig. 6E). Thus, it is likely that anagliptin suppressed LPS-induced NF-κB activation, thereby reducing the cytokine expressions in hepatic and adipose tissues.

DISCUSSION

In the present study, anagliptin was demonstrated to exert a direct anti-inflammatory effect on macrophages, as evidenced by suppressed expressions of the mRNA for inflammatory cytokines such as TNFα, IL-1β, IL-6, and IL-12 in response to LPS or palmitate (Fig. 1). Interestingly, the protein expression level of DPP-IV in RAW264.7 cells was so low that our immunoblotting technique failed to detect it. In fact, the measurement of enzymatic activity revealed DPP-IV activity in the cell lysates of RAW264.7 cells to be lower than those in mouse renal and hepatic tissues and in peritoneal macrophages as well as in the pancreatic, thoracic aorta, and 3T3-L1 cells examined in our experiments (Fig. 2A). These data may raise doubts as to whether the anti-inflammatory effect of anagliptin is actually attributable to DPP-IV inhibition or instead to other function(s) induced via its specific structure. Thus, we examined the effects of another DPP-IV inhibitor, sitagliptin, and showed that this drug exhibited a similar anti-inflammatory action, albeit one weaker than that of anagliptin. The difference in the degrees of anti-inflammatory effects between anagliptin and sitagliptin may be explained by different inhibitory efficiencies against DPP-IV in cell lysates and supernatants, since the treatments with anagliptin and sitagliptin resulted in similar inhibitory effects on DPP-IV activity in the supernatants of both RAW and 3T3-L1, whereas in both cell lysates anagliptin more strongly inhibited DPP-IV activity than sitagliptin. DPP-IV activity in cell lysates indicates mainly the activity of DPP-IV expressed on the cell surface, whereas that in supernatants indicates the activity of the soluble form of DPP-IV (sDPP-IV).

Anagliptin was also shown to act on 3T3-L1 adipocytes; i.e., the inflammatory cytokine expressions induced by LPS or TNFα were suppressed. This effect appears to be weaker for 3T3-L1 adipocytes (Fig. 3) than for RAW264.7 cells. In the coculture system of RAW macrophages and 3T3-L1 adipocytes stimulated with LPS, the effects of anagliptin on the suppression of cytokine expressions in 3T3-L1 adipocytes were enhanced compared with the stimulation of either RAW macrophages or 3T3-L1 adipocytes alone and were evident at the 10 μM concentration. This indicated a clearly inhibitory effect, reflecting the combined bidirectional actions and accumulated anti-inflammatory effects on macrophages and adipocytes over the time course. Additionally, the reduction of LPS-stimulated TNFα production from macrophages by anagliptin might have been the main contributor to this effect because the anti-inflammatory action was dose dependent, as shown in Fig. 4. Thus, it is very likely that anagliptin exerts effects on both macrophages and adipocytes, which in turn leads to synergistic effects.

In addition to the in vitro data, this study also demonstrated hepatic NF-κB activation as well as cytokine expressions to be suppressed by anagliptin treatment in the livers and adipose tissue of mice infused with LPS. The data showing reduced serum concentrations of cytokines raise the possibility of the anti-inflammatory effect of anagliptin not to being limited to specific tissues but rather to being present throughout the body.

To date, anti-inflammatory effects of DPP-IV inhibitors as well as GLP-1 have been reported in various tissues, including...
the liver, pancreatic β-cells, vascular cells associated with atherosclerotic lesions, adipose tissue, and the kidney (8, 7, 26, 28, 38, 50). An increased serum GLP-1 concentration was suggested to be one of the underlying mechanisms, and GLP-1 does reportedly contribute to the prevention of cardiovascular events (41). Understandably, serum active GLP-1 was elevated from ~12 to 25 pg/ml by administration of anagliptin (Fig. 6E). It is conceivable that serum-active GLP-1 elevation contributed to if it was not entirely responsible for suppression of inflammatory cytokine levels in LPS-treated mice. However, the evidence obtained in this study showing the in vitro suppressive effects on cytokine production in RAW cells by anagliptin and sitagliptin suggests the anti-inflammatory effects of these DPP-IV inhibitors to be direct actions rather than via increased concentrations of incretins such as GLP-1 or GIP. The number of reported substrates of DPP-IV, which is a serine protease, exceeds 80 (13, 24).

Among numerous DPP-IV targets, stromal cell-derived factor-1α (SDF-1α; widely known as CXCL12) is expressed in adipocytes, monocytes, and perivascular macrophages (7, 37) and reportedly exerts anti-inflammatory effects in mice via suppression of LPS-induced TNFα and IL-6 productions by macrophages (7). CXCR4, a receptor for SDF-1α, is expressed on adipocytes and macrophages (47). Thus, one possibility is that DPP-IV inhibitors prevent DPP-IV-induced inactivation of SDF-1α such that SDF-1α exerts an inhibitory effect on LPS-induced inflammation.

Another possible mechanism involves sDPP-IV lacking the transmembrane domain and intracellular tail (13). The interaction with sDPP-IV induces the phosphorylation of cavelolin-1 (30), thereby reducing complex formation between Toll-interacting protein (Tollip) and IL-1 receptor-associated serine/threonine kinase 1 (IRAK-1), which leads to the activation of NF-κB via an IRAK-1-dependent mechanism (29). Some re-
ports have indicated that Tollip inhibits IRAK-1 activation by associating with IRAK-1 (27, 34, 49), and another report demonstrated that Tollip serves as a suppressor of Toll-like receptor (TLR) signaling (23). Taking into consideration that Tollip and IRAK-1 are major components of the TLR signaling pathway (36), DPP-IV inhibitors may interfere with LPS-mediated TLR4 signal pathway transduction through inhibition of sDPP-IV, and anagliptin may consequently exert an inhibitory effect on NF-κB activation.

Regarding the role of DPP-IV inhibitors in TNFα-induced expressions of inflammatory cytokines, caveolin-1 binds to TNF receptor-associated factor 2 (TRAF2), and the strength of this binding triples with the phosphorylation of caveolin-1, thereby enhancing TRAF2 stability (4). The caveolin-1-TRAF2 complex associates transiently with the TNF receptor-associated death domain and receptor-interacting protein in response to TNFα stimulation, and signal transduction consequently induces activation of NF-κB and AP-1 (3, 11). Considering that TRAF2 stability accounts for half of TRAF2 and Tollip and IRAK-1 are major components of the TLR signaling (23). Taking into consideration that TRAF2 stability accounts for half of TRAF2 and Tollip inhibits IRAK-1 activation by associating with IRAK-1 (27, 34, 49), and another report demonstrated that Tollip serves as a suppressor of Toll-like receptor (TLR) signaling (23). Taking into consideration that Tollip and IRAK-1 are major components of the TLR signaling pathway (36), DPP-IV inhibitors may interfere with LPS-mediated TLR4 signal pathway transduction through inhibition of sDPP-IV, and anagliptin may consequently exert an inhibitory effect on NF-κB activation.

In conclusion, our study clearly demonstrated that anagliptin improves inflammation in adipose as well as hepatic tissues by affecting the stability of TRAF2 indirectly. Further study is necessary to clarify these issues.

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