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ORIGINAL RESEARCH The $I\alpha$, 25(OH)₂D₃ Analogs ZKI 59222 and ZK191784 Show Anti-Inflammatory Properties in Macrophage-Induced Preadipocytes via Modulating the NF- κ B and MAPK Signaling

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Purpose: Key research findings suggest that attenuating metaflammation in adipose tissue might be a strategic step to prevent the metabolic syndrome and its associated disease outcomes. The anti-inflammatory effects of $1\alpha_2$ (OH)₂D₃ have been confirmed in our previous studies, but adverse effects induced at high concentrations restrict its potential clinical translation. Two synthetic 1a,25(OH)₂D₃ analogs ZK159222 and ZK191784 have manifested promising tissuespecific immunomodulatory actions, but limited data are available on adipose tissue. Hence, in this study, we investigated whether ZK159222 and ZK191784 act on preadipocytes or macrophages to attenuate metaflammatory responses via modulating inflammatory and metabolic signaling in macrophage-induced preadipocytes.

Methods: Preadipocyte-specific effects of ZK159222 and ZK191784 on macrophage-induced preadipocytes were tested by pre-incubating and incubating preadipocytes with the analogs and MacCM. Separately, macrophage-specific effects of both analogs on macrophage-induced preadipocytes were tested by incubating preadipocytes with analog-MacCM or MacCM. The effects of 1a,25(OH)₂D₃ were also examined and set as the positive control. Metaflammatory responses were determined as the concentrations and gene expression of major pro-inflammatory cytokines including IL-1B, IL-8, IL-8, MCP-1 and RANTES, measured using ELISA and qPCR. Inflammatory and metabolic signaling including NF-KB and MAPK were probed using Western blotting.

Results: ZK159222 and ZK191784 act on preadipocytes and macrophages to decrease the secretion and gene expression of the major pro-inflammatory cytokines in macrophage-induced preadipocytes. The anti-inflammatory effects were at least as potent as $1\alpha_2 (OH)_2 D_3$, and no preadipocyte apoptosis was induced at high concentrations. In addition, mostly at high concentrations, both analogs moderately decreased the phosphorylation of relA, p44/42 and p38 MAPK in macrophage-induced preadipocytes.

Conclusion: ZK159222 and ZK191784 act on macrophages and preadipocytes to attenuate metaflammatory responses in macrophage-induced preadipocytes, by decreasing phosphorylation of relA/NF-kB, p44/42 and p38 MAPK.

Keywords: vitamin D, metaflammatory response, relA, p44/42, p38

Introduction

The metabolic syndrome (MetS) is a constellation of interrelated risk factors including central obesity, insulin resistance, dyslipidemia and hypertension, which have been considered to promote the development of type 2 diabetes, cardiovascular disease, and

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even a plethora of cancers.^{1,2} A growing body of evidence suggests that metaflammation in adipose tissue contributes to the onset and progress of MetS.^{3,4} Metaflammation is mainly characterized as adipose tissue expression of various proinflammatory cytokines stimulated by M1 macrophage infiltration.⁵ Therefore, attenuating adipose tissue metaflammation might be a strategic step to prevent MetS and its associated disease outcomes.

The immunomodulatory properties of 1α ,25(OH)₂D₃ have suggested it may have a role in treating inflammatory diseases.⁶ 1α ,25(OH)₂D₃ has also long been known to influence metabolic and immune functions of adipose tissue.⁷ Moreover, the anti-inflammatory properties of 1α ,25(OH)₂D₃ in adipose tissue metaflammation were confirmed in our previous studies.^{8,9} However, adverse effects, i.e. calcium release-activated apoptosis, potentially restrict its clinical translation.^{8,10} Thus, during recent decades, attempts have been made to synthesize 1α ,25 (OH)₂D₃ analogs with preservation of immunomodulatory properties but elimination of calcium homeostasis disruption.¹¹

In this study, we primarily investigated whether two synthetic 1α ,25(OH)₂D₃ analogs (namely ZK159222 and ZK191784) act on preadipocytes or macrophages to attenuate metaflammatory responses in macrophage-induced preadipocytes. In addition, both inflammatory and metabolic signaling including NF- κ B and mitogenactivated protein kinase (MAPK) were probed to elucidate the underlying anti-inflammatory mechanisms.

Materials and Methods Reconstitution of ZK159222, ZK191784 and $I\alpha$,25(OH)₂D₃

ZK159222, ZK191784 (kindly provided by Bayer AG, Germany) and 1α ,25(OH)₂D₃ (ENZO Life Sciences, USA) were reconstituted in dimethyl sulfoxide to a concentration of 2.4×10^{-5} M and then diluted in media (indicated as below) to final concentrations of 10 nM and 1 μ M.

Macrophage Culture and Stimulation

The human THP-1 monocytic cell line was kindly provided by Professor Helen R Griffiths (Aston University, UK) and cultured in RPMI-1640 medium (Sigma-Aldrich, UK) with 10% fetal bovine serum at 37°C with 5% CO₂. Upon reaching a cell density of 1×10^6 cells/mL, supernatants were replaced with 100 nM phorbol 12-myristate 13acetate (Sigma-Aldrich, UK) in RPMI-1640 medium with

10% fetal bovine serum to differentiate the monocytes to macrophages for 48 h. Subsequently, supernatants were aspirated and 1 µg/mL lipopolysaccharide in RPMI-1640 medium was added to polarize the macrophages to proinflammatory M1 dominant phenotype.¹² The M1 dominant macrophages were cultured in RPMI-1640 medium for macrophage-conditioned medium (MacCM), or incubated with ZK159222 (10 nM and 1 µM), ZK191784 (10 nM and 1 μ M) or 1 α ,25(OH)₂D₃(10 nM) in RPMI-1640 medium for VD-MacCM. All the supernatants were collected after 24 h, filtered through 0.22 µm filters and stored at -80°C (the THP-1 cell line was originally purchased by Professor Helen Griffiths in Birmingham from the UK Government laboratory in Porton Down that has ethical approval for the development of such cell lines. For specific details, please refer to Metabolic memory effect of the saturated fatty acid, palmitate, in monocytes (DOI: 10.1016/j.freeradbiomed.2012.05.026), which was published in 2009 by Professor Griffiths' research group, firstly described the THP-1 cell line as having been purchased from Health Protection Agency Culture Collections (Porton Down, Salisbury, UK); all RPMI-1640 media used in culture or stimulation were supplemented with 1% penicillin/streptomycin).

Preadipocyte Culture and Stimulation

Commercially available human white preadipocytes derived from subcutaneous adipose tissue of a 44 years old female Caucasian subject with a body mass index of 21 kg/m² (PromoCell, Germany), were cultured to confluence as previously described.9 Following this, supernatants were aspirated and the preadipocytes were pre-incubated in preadipocyte growth medium (PromoCell, Germany) or with ZK159222 (10 nM and 1 μ M), ZK191784 (10 nM and 1 μ M) or 1 α ,25 (OH)₂D₃ (10 nM) in preadipocyte growth medium for 48 h. Subsequently, supernatants were aspirated and the preadipocytes were incubated with 25% RPMI-1640 medium (the control) or 25% MacCM or 25% MacCM along with ZK159222 (10 nM and 1 µM), ZK191784 (10 nM and 1 μ M) or 1 α ,25(OH)₂D₃ (10 nM) in preadipocyte growth medium for 24 h. Preadipocyte and supernatant collection was conducted after the incubation. Separately, when confluence was reached, supernatants were aspirated and the preadipocytes were incubated with 25% RPMI-1640 medium (the control), 25% MacCM or VD-MacCM (indicated as above) in preadipocyte growth medium for 24 h before preadipocyte and supernatant collection (all preadipocyte growth media used in culture or stimulation were supplemented with 100 U/mL penicillin, 100 $\mu g/mL$ streptomycin and 0.25 $\mu g/mL$ amphotericin B).

Measurement of Metaflammatory Responses

Proteins were extracted from the preadipocytes, and content measured as previously described.¹³ The concentrations of interleukin (IL)-1 β , IL-6, IL-8, monocyte chemoattractant protein (MCP)-1 and regulated on activation, normal T cell expressed and secreted (RANTES) in the supernatant were measured independently in duplicate using human ELISA kits following the manufacturer's instructions (R&D Systems, UK) and SPECTROstar Nano Microplate Reader (BMG LABTECH, Germany), normalized to the total cell protein and presented as ng (cytokine)/mg(cell protein).

RNA was extracted from Trizol-lysed preadipocytes and converted to cDNA using cDNA synthesis kit (Bio-Rad, UK). The relative gene expression of IL-1 β , IL-6, IL-8, MCP-1 and RANTES were measured as Ct value independently in duplicate using TaqMan gene expression assays (Applied Biosystems, UK), qPCR core kit following the manufacturer's instructions (Eurogentec, Belgium) and Stratagene Mx3005P instrument system, normalized to the internal reference PPIA,¹⁴ and presented as fold change relative to control using the 2^{- $\Delta\Delta$ ct} formula.¹⁵

Western Blotting

Proteins were extracted from the preadipocytes and measured as previously described.¹³ The intracellular densities of relA, phosphorylated relA, p44/42 MAPK, phosphorylated p44/42 MAPK, p38 MAPK and phosphorylated p38 MAPK were measured using the method previously described,¹³ and normalized to the internal control vinculin. All the antibodies used (New England BioLabs; Abcam, UK) were diluted according to the manufacturer's instructions. The phosphorylation level of relA, p44/42 MAPK and p38 were calculated as the ratio of phosphorylated relA to relA, phosphorylated p44/42 MAPK and phosphorylated p38 MAPK to p38 MAPK, respectively, and presented as fold change relative to control.

Statistical Analysis

Data were analyzed using one-way ANOVA and followed by Tukey's test for individual comparison (GraphPad Prism 5, USA). A value of P<0.05 was regarded as statistically

significant. The results were confirmed by three independent experiments and shown as mean \pm SEM.

Results

ZK159222 and ZK191784 Act on Preadipocytes to Reduce the Major Pro-Inflammatory Cytokines Secreted from Macrophage-Induced Preadipocytes

Metaflammatory responses were stimulated by inducing preadipocytes with 25% MacCM. IL-1 β , IL-6, IL-8, MCP-1 and RANTES were selected as major pro-inflammatory cytokines expressed in macrophage/MacCM-induced preadipocytes in keeping with published studies.^{16–21} The ELISA results show that secretion of the major pro-inflammatory cytokines was increased by 13 ~ 387-fold from MacCMinduced preadipocytes (Figure 1A).

To test whether ZK159222, ZK191784 or 1α ,25(OH)₂D₃ act on preadipocytes to attenuate the metaflammatory responses in macrophage-induced preadipocytes, preadipocytes were pre-incubated with ZK159222, ZK191784 and 1α ,25(OH)₂D₃ to boost their efficacy as formally established,¹³ and then incubated with ZK159222, ZK191784 and 1α ,25 (OH)₂D₃ along with induction of MacCM.

In accordance with our previous study,⁹ 10 nM of 1 α ,25 (OH)₂D₃ generally reduced the major pro-inflammatory cytokines secreted from MacCM-induced preadipocytes by 43 ~ 81%. 10 nM of ZK159222 and ZK191784 also exhibited significant anti-inflammatory effects as the overall pro-inflammatory secretions from MacCM-induced preadipocytes were decreased by 28 ~ 69% and 27 ~ 68%, respectively. Tukey's test indicates that both synthetic analogs reduced the secretion of the major pro-inflammatory cytokines as effectively as 1 α ,25(OH)₂D₃ (Figure 1A).

Preadipocytes started to undergo apoptosis in 2–4 h when pre-incubated with 1 μ M of 1 α ,25(OH)₂D₃ (data not shown). However, 1 μ M of ZK159222 and ZK191784 significantly and similarly reduced the overall pro-inflammatory secretion from MacCM-induced preadipocytes by 23 ~ 67% and 29 ~ 69% (Figure 1A).

ZK159222 and ZK191784 Act on Preadipocytes to Inhibit the Major Pro-Inflammatory Cytokines Expressed in Macrophage-Induced Preadipocytes

The qPCR results show that gene expression of the major pro-inflammatory cytokines was increased by $13 \sim 1933$ -





Figure I Continued.

fold in MacCM-induced preadipocytes. Consistent with our previous study,⁹ 10 nM of 1α ,25(OH)₂D₃ universally inhibited the major pro-inflammatory cytokines expressed in MacCM-induced preadipocytes by 61 ~ 88%. Likewise, 10 nM of ZK159222 and ZK191784 decreased the overall pro-inflammatory gene expression in MacCM-induced preadipocytes by 31 ~ 68% and 67 ~ 89%, though ZK159222 appeared less potent. Moreover, 1 μ M of ZK159222 and ZK191784 similarly inhibited the overall pro-inflammatory gene expression by 47 ~ 74% and 57 ~ 76% (Figure 1B).

ZK159222 and ZK191784 Act on Preadipocytes to Decrease Phosphorylation of Inflammatory and Metabolic Signaling in Macrophage-Induced Preadipocytes

During adipose tissue metaflammation, the NF- κ B signaling is activated via phosphorylation of the transcription factor relA in

the nucleus.^{22,23} The Western blotting results show that intracellular levels of relA were not affected, but the phosphorylation was increased by 1.2-fold in MacCM-induced preadipocytes. In accordance with our previous study,⁹ 10 nM of 1α ,25(OH)₂D₃ decreased relA phosphorylation by 39% in MacCM-induced preadipocytes. Although relA phosphorylation was not affected by 10 nM of ZK159222 or ZK191784, 1 µM of both synthetic analogs significantly and consistently decreased the phosphorylation by 17% and 17%, respectively (Figure 2A and B) (for MacCM-induced preadipocytes coincubated with 1a,25(OH)₂D₃, ZK159222 and ZK191784, the quantifications were calculated the as percentages of $\frac{\text{the relative fold changes of}(MacCM-induced preadipocytes-the co-incubation}){\text{the relative fold changes of MacCM-induced preadipocytes}}).$

The MAPK signaling are required for physiological metabolic adaptation, but inappropriate activation has been associated with the development of MetS.²⁴ As conventional MAPKs, p44/42 and p38 are activated via phosphorylation in the cytoplasm.²⁵ In this study, though intracellular levels of p44/42 and p38 MAPK were not affected, the phosphorylation of both MAPKs in MacCM-induced preadipocytes were



Figure 1 Preadipocyte-specific effects of ZK159222, ZK191784 and 1α ,25(OH)₂D₃ on the major pro-inflammatory cytokines secreted and expressed in macrophage-induced preadipocytes. Preadipocytes were pre-incubated in preadipocyte growth medium or with ZK159222 (10 nM and 1 μ M), ZK191784 (10 nM and 1 μ M) or 1α ,25 (OH)₂D₃ (10 nM) in preadipocyte growth medium for 48 h, and then incubated with 25% RPMI-1640 medium (the control) or 25% MacCM or 25% MacCM along with ZK159222 (10 nM and 1 μ M), ZK191784 (10 nM and 1 μ M) or 1α ,25(OH)₂D₃ (10 nM) in preadipocyte growth medium for a further 24 h. Supernatants and preadipocytes were collected after the incubated A) The concentrations of (a) IL-1 β , (b) IL-6, (c) IL-8, (d) MCP-1 and (e) RANTES were measured using ELISA. (Panel B) The relative gene expression of (a) IL-1 β , (b) IL-6, (c) IL-8, (d) MCP-1 and (e) RANTES were measured using one-way ANOVA. The results are shown as means ± SEM for groups of 6 and confirmed by 3 independent experiments. A significant difference to control is indicated by ***(p<0.001); to MacCM by # (p<0.05), ##(p<0.01) and ####(p<0.001); among ZK159222, ZK191784 and 1 α ,25(OH)₂D₃ by &(p<0.05), &&(p<0.05), and &&(p<0.05).

increased by 0.2-fold and 1.9-fold, whereas 10 nM of 1α ,25 (OH)₂D₃ decreased p44/42 and p38 MAPK phosphorylation by 30% and 76%. For the analogs, only 1 μ M of ZK159222 de-phosphorylated p44/42 MAPK (by 31%) in MacCM-induced preadipocytes. By contrast, (10 nM and 1 μ M) both analogs universally decreased p38 MAPK phosphorylation by 27% to 58%, though ZK159222 appeared least effective (Figure 2A, C and D).

ZK159222 and ZK191784 Act on Macrophages to Reduce the Major Pro-Inflammatory Cytokines Secreted and Expressed in Macrophage-Induced Preadipocytes

To test whether ZK159222, ZK191784 or $1\alpha, 25({\rm OH})_2D_3$ (VD) also act on macrophages to attenuate the

metaflammatory responses in macrophage-induced preadipocytes, preadipocytes were incubated with VD-MacCM. VD-MacCM were produced by incubating macrophages with ZK159222, ZK191784 and 1α ,25 (OH)₂D₃.

Compared with MacCM-induced preadipocytes, (10 nM) 1α ,25(OH)₂D₃-MacCM decreased secretion of the major pro-inflammatory cytokines by 25 ~ 50%, with the exception of IL-1 β . Likewise, (10 nM) ZK159222-MacCM and ZK191784-MacCM similarly reduced the major pro-inflammatory secretion by 33 ~ 54% and 23 ~ 51%. Furthermore, (1 μ M) ZK159222-MacCM and ZK191784-MacCM indistinguishably decreased the overall pro-inflammatory secretion by 30 ~ 63% and 24 ~ 44%, respectively (Figure 3A).

In parallel, (10 nM and 1 μ M) ZK159222-MacCM, (10 nM and 1 μ M) ZK191784-MacCM and (10 nM) 1 α ,25



Figure 2 Preadipocyte-specific effect of ZK159222, ZK191784 and $I_{\alpha,25}(OH)_2D_3$ on inflammatory and metabolic signaling in macrophage-induced preadipocytes. Preadipocytes were pre-incubated in preadipocyte growth medium or with ZK159222 (10 nM and 1 μ M), ZK191784 (10 nM and 1 μ M) or $I_{\alpha,25}(OH)_2D_3$ (10 nM) in preadipocyte growth medium for 48 h, and then incubated with 25% RPMI-1640 medium (the control) or 25% MacCM or 25% MacCM along with ZK159222 (10 nM and 1 μ M), XK191784 (10 nM and 1 μ M) or $I_{\alpha,25}(OH)_2D_3$ (10 nM) in preadipocyte growth medium for a further 24 h. Preadipocytes were collected after the incubation. (**A**) The intracellular densities of relA, phosphorylated relA, p44/42 MAPK, phosphorylated p44/42 MAPK, p38 MAPK, phosphorylated p38 MAPK and vinculin were measured using Western blotting. The phosphorylation levels of (**B**) relA, (**C**) p44/42 MAPK and (**D**) p38 MAPK are shown. Data were analyzed using one-way ANOVA. The results are shown as means ± SEM for groups of 3 and confirmed by 3 independent experiments. A significant difference to control is indicated by *(p<0.05) and ***(p<0.001); to MacCM by ##(p<0.01) and ###(p<0.001); among ZK159222, ZK191784 and $I_{\alpha,25}(OH)_2D_3$ by &&(p<0.01) and &&&(p<0.001).





Figure 3 Continued.



Figure 3 Macrophage-specific effect of ZK159222, ZK191784 and 1α ,25(OH)₂D₃ on the major pro-inflammatory cytokines secreted and expressed in macrophage-induced preadipocytes. Preadipocytes were incubated with 25% RPMI-1640 medium (the control), 25% MacCM or VD-MacCM in preadipocyte growth medium for 24 h. Supernatants and preadipocytes were collected after the incubation. (Panel **A**) The concentrations of (**a**) IL-1 β , (**b**) IL-6, (**c**) IL-8, (**d**) MCP-1 and (**e**) RANTES were measured using ELISA. (Panel **B**) The relative gene expression of (**a**) IL-1 β , (**b**) IL-6, (**c**) IL-8, (**d**) MCP-1 and (**e**) RANTES were measured using one-way ANOVA. The results are shown as means ± SEM for groups of 6 and confirmed by 3 independent experiments. A significant difference to control is indicated by ***(p<0.001); to MacCM by #(p<0.05), ##(p<0.01) and ####(p<0.001); among VD-MacCM by &(p<0.05), &&(p<0.01) and &&&(p<0.01).

(OH)₂D₃-MacCM inhibited the overall pro-inflammatory gene expression by 25 ~ 52% and 33 ~ 59%, 34 ~ 78% and 41 ~ 67%, 39 ~ 76%, respectively. The overall anti-inflammatory effects achieved were largely similar, though (10 nM) ZK191784-MacCM appeared most potent in inhibiting the gene expression of IL-8 and RANTES, while least effective on IL-1 β expression (Figure 3B).

Discussion

Physiologically, macrophage–preadipocyte interactions influence especially adipogenesis, lipolysis and apoptosis, which are fundamental metabolic processes to maintain adipose tissue homeostasis.^{26,27} During metaflammation, infiltration of excess M1 macrophages mobilize preadipocytes in paracrine/autocrine manners to disrupt adipose tissue

homeostasis, which might make a critical contribution to MetS.^{3,4,27,28} Hence, in this study, the metaflammatory responses were stimulated in macrophage-induced preadipocytes and measured only in supernatant/lysate extracted from the preadipocytes, since they are the progenitor of adipose tissue, rather than macrophages.²⁷ The current results revealed that secretion and gene expression of the major pro-inflammatory cytokines were significantly enhanced in macrophage-induced preadipocytes.

 1α ,25(OH)₂D₃ has been shown to improve inflammatory as well as metabolic biomarkers of MetS,²⁹ but there still is a long way to its clinical translation, which may be dependent upon the development of advantageous synthetic analogs and research to unravel their immunometabolic effects and mechanism of action.³⁰ The 1α ,25(OH)₂D₃ analogs ZK159222 and ZK191784 have manifested a number of tissue-specific immunomodulatory actions,³¹⁻³³ but limited data are available on adipose tissue. In this study, preadipocyte and macrophage-specific effects of ZK159222 and ZK191784 on metaflammatory responses in macrophage-induced preadipocytes were thoroughly explored. 1α , $25(OH)_2D_3$ served as the positive control since we previously demonstrated that it acts on preadipocytes to attenuate the metaflammatory responses in macrophage-induced preadipocytes.⁹ The present results show that ZK159222 and ZK191784 act on preadipocytes and macrophages to attenuate the metaflammatory responses in macrophage-induced preadipocytes (Figure 4). Although some differences in effects were obvious, both synthetic analogs not only decreased secretion and gene expression of the major pro-inflammatory cytokines in macrophage-induced preadipocytes as effectively as 1a,25(OH)₂D₃, but also displayed distinct anti-inflammatory advantages at high concentrations, for preadipocyte apoptosis was not induced. Limitations of this study include the fact that we focused our assessment of the metaflammatory response on proinflammatory cytokines. The cytokine array results revealed that 1α ,25(OH)₂D₃ increased serpin-E1 secreted from macrophage-induced preadipocytes. Overexpression of serpin-E1 has been suggested to show some beneficial effects on adipose tissue in metabolic syndrome.³⁴ However, IL-1RA, which acts as the inhibitor of IL-1 β ,³⁵ was paradoxically decreased (Supplementary Figure). Therefore, the effects of the synthetic analogs on anti-inflammatory cytokines during adipose tissue metaflammation should be considered in the future.

As a crucial inflammatory signaling activated during metaflammation, the NF- κ B/relA integrates with metabolism to disrupt adipose tissue homeostasis and contribute to MetS.^{22,36} Moreover, MAPK has been suggested to be an important metabolic signaling to provoke MetS when inappropriately activated.²⁴ Specifically, p44/42 and p38 MAPK function in adipose tissue to regulate adipogenesis and lipolysis, which are characteristically disordered in MetS.^{37–40} Only preadipocyte-specific modulating effects on NF- κ B and MAPK signaling in macrophage-induced preadipocytes were measured in this study, since



Figure 4 ZK159222 and ZK191784 show anti-inflammatory properties in macrophage-induced preadipocytes via modulating the NF- κ B and MAPK signaling, but their effects on adipose tissue homeostasis remain to be explored. White adipose tissue is composed of mature adipocytes and a stromal vascular fraction containing preadipocytes and macrophages. During metaflammation, excessive infiltration of M1 macrophages induces preadipocytes to secrete and express major pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, MCP-1 and RANTES, thereby contributing to disruption of adipose tissue homeostasis. Intriguingly, the synthetic 1α , 25(OH)₂D₃ analogs ZK159222 and ZK191784 act on preadipocytes and macrophages to attenuate the metaflammatory responses in preadipocytes, and the anti-inflammatory actions are exerted by de-phosphorylating inflammatory and metabolic signaling including reIA/NF- κ B, p44/42 and p38 MAPK. Moreover, macrophage–preadipocyte interactions influence adipogenesis, apoptosis and lipolysis to maintain adipose tissue homeostasis. Hence, in future studies, it would be interesting to investigate the effects of these analogs on the fundamental metabolic processes in adipose tissue.

not only preadipocytes are the progenitor of adipose tissue, but also were directly pre/incubated with the synthetic analogs.²⁷ The results revealed that the phosphorylation of relA, p44/42 and p38 MAPK were increased in macrophage-induced preadipocytes. 1α ,25(OH)₂D₃ acted on preadipocytes to decrease the phosphorylation of relA, p44/42 and p38 MAPK in macrophage-induced preadipocytes. ZK159222 and ZK191784 also de-phosphorylated these inflammatory and metabolic signaling, but most effectively when used at high concentrations. Last but not least, it would be interesting in future studies to investigate the effects of these analogs on those fundamental metabolic processes in adipose tissue (Figure 4).

Conclusion

The 1α ,25(OH)₂D₃ analogs ZK159222 and ZK191784 act on macrophages and preadipocytes to attenuate secretion and gene expression of the major pro-inflammatory cytokines in macrophage-induced preadipocytes, via decreasing phosphorylation of NF- κ B/relA, p44/42 and p38 MAPK.

Abbreviations

IL, interleukin; MacCM, macrophage-conditioned medium; MAPK, mitogen-activated protein kinase; MCP, monocyte chemoattractant protein; MetS, metabolic syndrome (MetS); RANTES, regulated on activation, normal T cell expressed and secreted.

Acknowledgments

Dr Chen Bing (formerly of the Institute of Ageing and Chronic Disease, University of Liverpool – now retired) co-conceived and co-designed the study, and provided ongoing advice during its conduct.

Author Contributions

JZ made substantial contributions to conception and design, acquisition of data. JW supervised the major findings. JW and JZ analyzed and interpreted the data. JZ drafted the article. JW revised it critically for important intellectual content. All the authors gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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Disclosure

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