Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia due to decreased insulin secretion, defective action or both. The ROSglut is one of the oral antidiabetic drugs used in type 2 DM and has a major role in the treatment of DM. However, RSG has a negative side effect on the bone as it stimulates the differentiation of bone marrow–mesenchymal stem cells (BM-MSCs) into adipocytes at the expense of osteoblasts in the bone marrow microenvironment, disturbing the normal balance of bone remodeling and causing BM adiposity. On the other hand, the trans-10,cis-12 conjugated linoleic acid (CLA), a fatty acid, is known as anti-adipogenic, pro-osteogenic. Therefore, this study was designed to assess whether CLA can alleviate the negative effect of RSG on bone. We used adipose tissue–derived mesenchymal stem cells (AT-MSCs) as a human in vitro model to study the effects of RSG and CLA and co-treatment (RSG+CLA) on the osteoblastic commitment and osteogenic differentiation of AT-MSCs. Osteoblastogenesis was assessed by Alizarin Red Staining and bone mineralization was assessed by Osteomage™ assays. Furthermore, adenosine was identified by Oil Red O Staining and LipidTOX™ assays. Besides, the level of expression of osteogenic and adipsogenic markers was measured on treated osteo- and adipodifferentiated MSCs using real time RT-PCR, immunohistochemistry (IHC) and western blot analysis. Compared to RSG group, the combined treatment group stimulates osteoblastogenesis, as evidenced by increased mineralization and upregulation of osteogenic markers OPN and RUNX2 and inhibits adipogenesis in osteogenic media as showed by decreased lipid content and downregulation of adipogenic markers FABP4, LPL, and adiponectin. In conclusion, the use of CLA as an adjunctive treatment reversed the effects of RSG on osteogenesis and adipsogenesis. Further preclinical and clinical studies will be undertaken to establish this treatment regimen for the successful treatment of diabetic patients with rosiglitazone without adverse side effects on bone.

Hypothesis and Objectives

Hypothesis: CLA co-treatment can reverse the balance of osteogenesis and adipogenesis in bone marrow microenvironment and counterbalance the side effects of RSG on bone.

Objectives: Successful use of adipose derived MSCs (AT-MSCs) to: (1) study the RSG-induced changes in the osteogenesis and adipogenesis in vitro and to (2) test the effect of CLA co-treatment in vivo. The study also aimed to: (1) determine the morphological effects of RSG and CLA on MSCs using microscopic techniques (optical microscopy, scanning electron microscopy, confocal microscopy), confocal microscopy and (2) study the gene expression modulation of linage specific markers and differentiation assays (e.g., Osteomage™ mineralization assay, Alizarin Red, and LipidTOX staining).

Results and Discussion

Effect on osteogenesis - Mineralization

Calcium deposition (Alizarin Red Staining)

Alizarin Red Quantification

(Arbitrary Unit)

Results: Calcium deposition significantly increased after addition of CLA and RSG+CLA to the osteoblastic–differentiated MSCs and led to higher percentage of calcium deposition than in the control and RSG alone treatment groups.

Effect on adipogenesis - Lipid Content

LipidTOX Staining

ImageJ LipidTOX Quantification

(Osteoblast Differentiation)

Normalized to Cell Count

Average Fl

(Arbitrary Unit)

Results: CLA and RSG+CLA treatment conditions appeared to show a trend of increase in lipogenic mineralization, although not statistically significant and the trend was more evident when normalized to cell counts.

Expression of Marker Proteins (IHC)

Gene Expression Tests of Osteogenic & Adipsogenic Markers (Real Time RT-PCR)

Levels of Osteogenic & Adipsogenic Markers (Western Blot Analysis)

Conclusion

- AT-MSCs can be used successfully as a human in vitro model to study the effect of different treatments on the adipogenic and osteogenic differentiation of MSCs.
- CLA co-treatment can reverse the side effects of RSG on bone remodeling and restore the balance between osteogenesis and adipogenesis (Stimulate osteoblastogenesis and inhibit adipogenic differentiation of MSCs in osteogenic medium).
- The use of CLA might alleviate the safety concerns regarding the deterioration of bone remodeling (BM adiposity) and the increased fracture risk associated with RSG treatment.
- The pre-clinical data regarding the in vivo effect of (RSG+CLA) on RSG-induced bone remodeling have to be investigated further as in vivo using animal models, and randomized clinical trials on T2DM patients.

References


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Conjugated Linoleic Acid (CLA) co-treatment alleviates antidiabetic drug, rosiglitazone associated deterioration of bone remodeling.

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