Methylglyoxal inhibited osteoblast mineralization and fracture healing

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INTRODUCTION
Diabetic patients have a higher fracture risk and a delayed fracture healing. This pathology has been explained by abnormal collagen cross-linking in bone tissue caused by AGEs (advanced glycation end products). Increased blood levels of methylglyoxal (MG), an intermediate derivative of glucose metabolism, in diabetic patients cause various systemic complications6,7, but the effect of MG on bone metabolism and bone healing has not been fully elucidated. In this study, we determined the effects of MG on bone formation using primary osteoblast cultures and a mouse tibia fracture model.

MATERIALS AND METHODS

In vitro osteoblast differentiation assay:
Osteoblasts were isolated from newborn (day 2) mouse calvaria. Cells were cultured in α-MEM with 10% FBS, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 µg/ml ascorbic acid, 10 mM β-glycerophosphate for the osteoblast differentiation assay. Cells were treated with MG (0 ~ 300µM) at 6 hour-intervals. On day 7, MTT, ALP activity, total collagen (estimated by hydroxyproline) assays and quantitative RT-PCR were assessed. On day 21, mineralized nodule formation assay (Alizarin red staining) and total collagen assay were performed.

Experimental Animals:
Nine-week-old male C57BL/6 mice were subjected to tibia fracture model with intramedullary pinning. After surgery, mice were divided into two treatment groups: MG (110mg/kg/day) and control (PBS). Daily intraperitoneal injection was performed for 2 weeks (Fig 2). At 14 days after fracture, mice were sacrificed, and microCT and biomechanical analyses were performed.

microCT:
Bone samples were scanned using the CosmoScan GX (Rigaku, Tokyo, Japan) at high resolution with a voxel size of 2.3 µm to image bone. An integration time of 300 ms, a current of 145 mA, and an energy setting of 55 kV were used. The threshold for bone tissue including callus was set to 3244 or higher, and external soft tissue including the bone marrow cavity and cortical bone were excluded.

Biomechanical Testing:
3-point bending test was performed using MZ-500S (MARUTO, Japan). The distance between the fulcrum points was set to 8 mm, the fracture site was placed in the center, and the fracture test was performed from above at a speed of 5 mm/min. A 500 N load cell was used.

Statistics:
Results are shown as the mean +/- standard deviation. Statistical tests included Student’s t-tests and two-way ANOVA followed by Tukey-Kramer test. p<0.05 was considered significant.

RESULTS

The effect of MG on cell viability and MG-H1 accumulation in mouse calvarial osteoblasts: We first measured the effect of MG on cell viability and proliferation in osteoblasts. MTT assay showed that MG concentrations up to 300 µM did not significantly reduce osteoblast viability or proliferation (data not shown).

The effect of MG on collagen matrix formation and mineralization in mouse calvarial osteoblasts: ALP activity was significantly reduced by MG treatment (Fig 1A). Alizarin red staining was also reduced by MG treatment (Fig 1B). No effect was observed on Collal gene expression and total collagen content by MG treatment (Fig 1C, D). Osteocalcin and Osteoprotegrin mRNAs expression or protein levels were induced by osteoblast, and their mRNA and protein levels decreased in a MG concentration-dependent manner (data not shown). Osterix is a master regulator of differentiation in primary osteoblasts was induced by osteoblast differentiation, and its expression level was markedly decreased in a MG concentration-dependent manner.

The effect of MG on mouse tibia fractures: microCT demonstrated that bone union was achieved in both groups at 2 weeks postoperatively (Fig. 2A). There was no difference in callus volume between the two groups (Fig 2B), but callus total mineral density (TMD) was significantly lower in the MG group; (Fig2 C), indicating that MG treatment inhibited callus mineralization. In the three-point bending test, stiffness and maximum force were not significantly different between the two groups (Fig 2D, E). However, failure time was significantly decreased in the MG group (Fig 2F), indicating a decrease in fracture callus strength due to MG treatment.

REFERENCE