Original

The Effect of High Glucose on Proliferation and Expression of Correlation Factors of MG63 Osteoblasts

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Abstract: The influence of high glucose on proliferation and expression of correlation factors including TNF related apoptosis inducing ligand (TRAIL), osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) of MG63 osteoblasts was detected in the present study. The relationship between the high concentration blood glucose and the physiological functions of human osteoblasts was studied. Then the pathogenesis of diabetes mellitus with chronic periodontitis was discussed. MG63 cells were cultured in different concentrations (5.5 mmol/l, 8 mmol/l, 11 mmol/l, 20 mmol/l, 30 mmol/l) medium for 24 hours. Cell proliferation was detected by MTT measurement. Flow cytometry was used to detect cell cycles. The mRNA expressions of TRAIL, OPG, and OPGL were detected by reverse transcription polymerase chain reaction (RT-PCR). The results showed that a low concentration of glucose has no significant influence on the proliferation of MG63 cells and other biological activity. With the increase of glucose concentration, the proliferation of MG63 cells was inhibited. The percentage of G1 phase cells was increased, the proportion of S phase kept unchanged and the percentage of G2 phase cells was decreased. With the increase of glucose concentration, the mRNA expressions of TRAIL and OPGL of MG63 cells showed an increasing trend while OPG showed an opposite trend. In conclusion, high glucose can inhibit the proliferation of MG63 and lead to the increasing mRNA expression of TRAIL and OPGL of human osteoblasts. However, the mRNA expression of OPG of human osteoblasts was decreased.

Key words: High glucose, MG63 cell, Cell cycle; Tumor necrosis factor

Introduction

High blood glucose concentration is harmful to many types of human cells, which causes many diseases, such as diabetes mellitus, stress and systemic inflammatory response after injury or operation¹⁾. Diabetes mellitus interact with various oral diseases. The abnormal blood glucose could cause periodontal, mucosal, endodontical and gland diseases of maxillofacial region²⁾. Effective control of glucose metabolism for diabetes mellitus patients is helpful to improve the periodontal inflammation and maintain periodontal health³⁾. It is known to all, diabetes mellitus patients are likely to develop osteoporosis⁴⁾. The alveolar bone is one of the most active parts of the human skeleton. The reconstruction of the alveolar bone is a complex physiological process controlled by the external signal, system hormone level and local factors. Alveolar bone resorption is the pathological change of bone reconstruction disorders caused by periodontitis, osteoporosis or orthodontics accidentally⁵⁾. These clinical issues affect people's oral health seriously. The present study is to detect the effect of

high glucose on the cell proliferation and cycle, the changes of TRAIL, OPG and OPGL expression. The high glucose concentration effect on the physiological function of human osteoblast was also determined. The pathogenesis of chronic periodontitis with diabetes mellitus was discussed to provide theory for orthodontic treatment.

Materials and Methods

Human osteoblast-like cells MG63 is provided by stomatology biology laboratory of Jilin University. MG63 were cultured in DMEM which contains 10% fetal bovine serum (FBS), 100 i/ml penicillin, and 100 ig/ml streptomycin at 37 °C and 5 % CO₂. The medium contains 5.5, 8, 11, 20 and 30 mmol/l glucose was changed every two days. MG63 cells in the experimental group were cultured in different medium.

MTT assay [3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide (MTT)] was used to estimate cell proliferation. MG63 cells were plated in 96-well plates at a density of 1×10³ cells/well and incubated at 37 °C for 24 hours. Then the medium contained glucose was used. MG63 cells were cultured for 24 hours. 20 µl MTT solution (5 mg/ml) was added to each

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Table 1. The influence of glucose concentration on the cell proliferation of MG63 cells

Glucose (mmol/l)	N	MTT (A value)	IR (%)
5.5	6	0.3138±0.0232	
8	6	0.3110±0.0334	0.09
11	6	0.2908±0.0120	7.33
20	6	0.2743±0.0137*	12.59
30	6	0.2643±0.0054*	15.78

*p < 0.05, compared with the results of 5.5 mmol/l group

well and cells were incubated at 37 °C for 4 hours. The medium was removed and 150 µl DMSO was added to each well. Absorbance (A) at 570 nm was detected. The average value was obtained to calculate growth inhibition. The cell growth inhibition rate was calculated as follows:

$$\text{Cell growth inhibition rate} = (1 - A_{\text{test}} / A_{\text{control}}) \times 100 \%$$

MG63 cells were cultured with glucose medium. After 24 hours, cells were harvested and washed with PBS. Cells were suspended in 500 µl of 70 % alcohol and kept at 4 °C for 2 hours. Then cells were washed with PBS, treated with 100 µl RNase A at 37 °C for 30 minutes and stained with 400 µl propidium iodide at 4 °C for 30 minutes. DNA was analyzed by flow cytometry. Cell cycle and apoptosis was analyzed using Milti Cycle Software. Each experiment was performed in triplicate.

The expression levels of the genes OPG, OPGL, TRAIL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined using RT-PCR. MG63 cells were seeded in 6-well plates. The total cellular RNA was extracted using TRIzol reagent in accordance with the manufacturer's instructions. GAPDH was used as an internal control. The primers used for PCR analysis were as follows: OPG forward, 5'-AGTGGGAGCAGAAGACATTG-3'; reverse, 5'-ATTGGACCTGGTTACCTATC-3'; OPGL forward, 5'-GCGTCGCCCTGTTCTTCTAT-3'; reverse, 5'-TTGGTGCTTCCTCCTTTCAT-3'; TRAIL forward, 5'-GGCATTTCATTCTGAGCAACTT-3'; reverse, 5'-GATCTCGTGATCTACCCACCTT3-3'; GAPDH forward, 5'-

GAAGGTGAAGGTCGGAGTC-3' reverse, 5'-GAAGATGGTGATGGGATTTC-3'.

Date was reported as means±standard deviations. LSD-t (least significant difference test) was used to compare means between two groups. One-way ANOVA was used to compare means of more than two groups. A value of P<0.05 was considered to be statistically significant. All experiments were repeated for three times.

Results

MTT assay showed that MG63 cell proliferation was inhibited with the increase of glucose concentration as shown in Table 1. In comparison with the control group, a significant decrease in proliferation (P<0.05) was observed with glucose at concentration 20 mmol/l and 30 mmol/l.

The cell cycle was evaluated by flow cytometry after the MG63 cells were exposed to glucose at various concentrations for 24 hours. As shown in Fig. 1 and Table 2, the cells in the G1 phase increased with the increase of glucose concentration. A reduction in the proportion of cells in G2 phase was observed. The results showed that glucose induced a cycle arrest in MG63 cell at G1 phase in a concentration-dependent manner. But there was no significant difference between 20 mmol/l and 30 mmol/l group, which showed that G1 phase inhibition was not obvious when the concentration of glucose reached a point.

RT-PCR results shown in Fig. 2 revealed that the OPG mRNA expression was down-regulated in the MG63 cell while the OPGL mRNA was up-regulated. And the TRAIL mRNA was also up-regulated by glucose in a concentration-dependent manner.

Discussion

The *in vitro* experiments were carried out to evaluate the influence of glucose concentration on the proliferation, cell cycle and biomineralization of MG63 cell. Different glucose concentrations were selected in the experiments which were generally considered a normal serum glucose level or be near the level after meal. For example, a glucose concentration of 5.5 mmol/l is equal to 99 mg/dl, which is generally supposed to normal serum glucose levels before meal. A glucose concentration of 8 mmol/l is equal to 144 mg/dl, which is supposed to normal serum

Table 2. The effect of glucose on cell cycle distribution of MG63

Glucose (mmol/l)	n	G1 phase (%)	S phase (%)	G2 phase (%)
5.5 mmol/l	3	40.9100±0.2945*	33.7967±0.9923	25.2933±0.9511*
8 mmol/l	3	49.1533±0.2279*	31.3167±0.6446	19.5300±0.6417*
11 mmol/l	3	52.2133±0.4792*	30.9233±0.1168	16.8633±0.3934*
20 mmol/l	3	56.2800±0.4330*	28.0700±0.6035*	15.6467±0.4054*
30 mmol/l	3	57.2467±0.3093*	30.5200±0.2889*	12.2333±0.3375*

*p < 0.05, compared with the results of 5.5 mmol/l group

Figure 1. The results of flow cytometry for different glucose concentration.

glucose levels two hours after meal. A glucose concentration of 11 mmol/l is equal to 196 mg/dl, which is supposed to serum glucose of uncontrolled diabetes mellitus. A glucose concentration of 20 mmol/l is equal to 360 mg/dl, which is supposed to serum glucose of diabetes mellitus with bone complication. 30 mmol/l

is considered to be high glucose media which is equal to 540 mg/dl.

Because MG63 cells display all of the characteristics of osteoblast while offer the advantages of being a high-purity tumor cell line with unlimited growth capacity. The cell line is widely used as an osteoblast model for basic research and pharmacodynamics observation⁶. Cell cycle plays an important role in cell growth regulation. Extensive attention was paid to interfering the cell growth process by changing the cell cycle.

In cell cycle, G1 phase is the preparation for protein synthesis DNA replication and Histone Synthesis is in S phase. There are two restriction points for cell cycle, one is at the change for G1 to s phase. The other is at the change for G2 to M phase. When cell is injured, cell cycle is blocked. This is conducive to damage repair, which would keep genetic stability. When injury is too serious to be repaired, cells will apoptosis.

Diabetes mellitus may promote fibroblast and osteoblast cell apoptosis. Through the animal experiment, Liu *et al.*⁷ found that apoptosis of osteoblasts and fibroblasts which were got from periodontal tissue of diabetic and periodontal rats increased. The number of osteoblasts decreased and bone formation reduced.

Figure 2. The influence of glucose concentration on TRAI, OPG and OPGL mRNA expression of MG63 cells.

inflammatory reaction of osteoblast. The current study yielded conflicting results about the effect by high serum glucose for biomineralization process of osteoblast. Weil et al¹⁾ found that high serum glucose concentration won't affect cytokine production and diffusion of bone marrow mesenchymal stem cells. García-Hernández et al.¹⁶⁾ found that high serum glucose concentration could change osteoblast biomineralization process of human. These conflicting results may be caused by different cell lines, experimental conditions and glucose concentrations. The mechanism is still unknown and expected to be further studied.

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