The Journal of Applied Pharmacology, 11, 81-84(2003)

High Glucose Inhibitory Effects on Bone Formation in UMR106 Cells

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(Received Mar. 18, 2003; Accepted Mar. 21, 2003)

Abstract–Recently, diabetes has been found to be associated with osteoporosis. Specially in IDDM In both type I and type II diabetes, glucose levels are elevated. Thus, a linkage between high glucose and osteoporosis can not be ruled out. In this study, an attempt has been made to observe the effect of high glucose on bone formation; osteoblast like UMR 106 cells were treated with high glucose (22 mM, 33 mM) for 1, 3 or 7 days. The high concentration of glucose inhibited markers of bone formation activity such as alkaline phosphatase and collagen synthesis. In addition, reduction in the level of total cellular protein in response to high glucose was also observed. This study showed high glucose concentration could alter the bone metabolism leading to a defective bone formation and thus paving the linkage of such situation to diabetic complications.

Keywords □ High glucose, Osteoblast, Bone resorption, Diabetes, Metabolic Bone Disease

Diabetes is diagnosed by glucose level, and the elevated extracellular glucose has shown to cause significant cellular effect like modulation of cellular redox, alteration in cell metabolic paths, activation of protein kinase C and glycosylation of protein and DNA. These changes lead to affect cell growth and function, and such situation has also been observed with acute change in bone turnover by glucose (Glowers *et al.*, 2002). In bone metabolism, mostly two type of functional bone cells are involved: osteoblast, the bone forming cells and osteoclast, responsible for bone resorption leading to bone loss. Under high glucose, which is also a situation in IDDM, many studies have demonstrated low mineral density in patients (Mc Nair 1979,1988 and shore *et al.*, 1981). In Japanese study 21% of patients have been found to have osteopenia in NIDDM (Imuia *et al.*, 1987). The occurrence of diabetic osteopenia has been linked to insulin deficiency and hyperglycemia (Terada *et al.*, 1998). Insulin also has stimulating effect on bone forming cells osteoblast (Canalis 1983) and hyperglycemia. High glucose situation, a principle characteristic of diabetes, has influence in many cellular function (Chio *et al.*, 1996), including accelerating of urinary Ca^{2+}, exaction in association with glycosuria and impairment of vitamin D metabolism that leads to Ca^{2+} malabsorption (McNair et 1988). All these observation leads to believe that it may be possible that a high glucose content might impair the bone metabolism, especially the bone forming process. Thus, to verify such possibility, in the present study we determine the effect of elevated glucose concentration on bone forming cells, osteoblast, using osteoblast like UMR106 cells, and these studies demonstrated that high glucose concentration has inhibitory effects on bone formation.

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MATERIALS AND METHODS

Cell Culture

Osteoblast-like UMR106 cells were grown in DMEM supplemented with 10 % FBS and antibiotics (100 u/ml penicillin - 100 μg/ml streptomycin) in a humidified atmosphere of 97 % air and 7 % CO_2. To investigate the effect of glucose on cells, equal numbers of UMR106 cells were seeded into 12-well plates. After cells were complimented (70 80%), washed three times with phosphate-buffered saline (PBS) and the medium was changed to medium of various concentration of glucose (5.5, 22 and 33 mM) or in osmotic medium (16.5 and 27.5 mM mannitol plus 5.5 mM glucose). For experiment, the cells were cultured for 1, 3 and 7 days and washed three times with PBS. The cells were detached from the plates with 0.1% triton X-100, and we collected cell suspensions. After sonication for 10 seconds, the cell suspensions were centrifuged at 1500 xg for 5 minutes at 4. The supernatant was used for the assay.

Alkaline Phosphatase activity

An alkaline phosphatase activity was carried out using ALP-K kit (YeongDong Pharmaceurical Corp.). The activity was measured in a spectrophotometer at 570 nm wavelength.

Collagen Synthesis

Measurement of collagen synthesis was performed by using Sircol Collagen assay kit (Biocolor Ltd.). The activity was measured in a spectrophotometer at 540 nm wavelength.

Protein Assay

Protein assay was measured with the method of Lowry *et al.*(1951).

Statistical Analysis

Data are expressed as the mean ± SD. The significance of differences between mean values was evaluated by Student's t-test.

RESULTS

Alkaline Phosphatase Activity

The exposure of UMR106 cells to high glucose (22 mM and 33 mM) led to a significant inhibition of alkaline phosphatase activity as compared to normal glucose (5.5 mM). The exposure of cells to 22 mM and 33 mM glucose concentration for 1 day showed inhibition of 38% and 61%, respectively. The exposure of cells for 3 days showed inhibition of 46% and 66% in each high glucose concentration. And the cells cultured for 7 days showed inhibition of alkaline phosphatase activity to 47% and 73% (Fig. 1, Fig. 2). These results revealed that the exposure of cells in high glucose concentration could inhibit alkaline phosphatase activity. When the cells were exposed to 16.5 mM mannitol plus 5.5 mM glucose, alkaline phosphatase activities were significantly inhibited in 1 day incubation, whereas in 3 days and 7 days incubation mannitol had little effects. On the other hand, when the cells were exposed to 27.5 mM mannitol plus 5.5 mM glucose for 1, 2 or 7 days, alkaline phosphatase activities were significantly inhibited. However, mannitol had lower inhibitory action as compared to glucose (Fig. 1, Fig. 2).

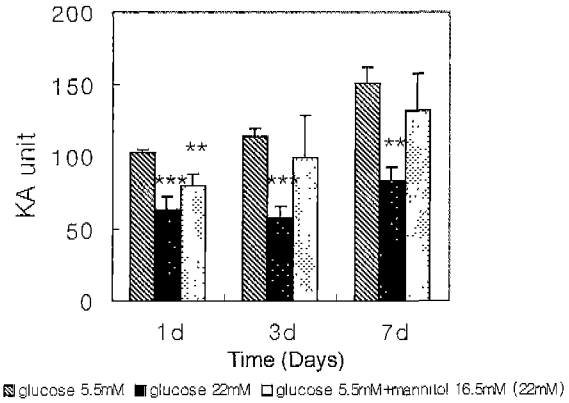

Fig. 1. Alkaline phosphatase activity in high glucose concentration (22 mM) on UMR106 cells. Cells were cultured in 5.5 mM glucose, 22 mM glucose and 5.5 mM glucose + 16.5 mM mannitol for 1 day, 3 days and 7 days. The medium was replaced with the same fresh medium on 3 days. Data are shown as the mean ± S.D. **: $p < 0.01$, ***: $p < 0.001$ ($n = 4$).

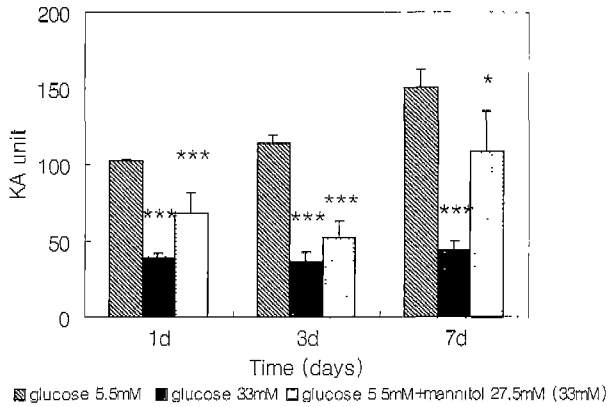

Fig. 2. Alkaline phosphatase activity in high glucose concentration (33 mM) on UMR106 cells. Cells were cultured in 5.5 mM glucose, 33 mM glucose and 5.5 mM glucose + 27.5 mM mannitol for 1 day, 3 days and 7 days. The medium was replaced with the same fresh medium on 3 days. Data are shown as the mean ± SD. *: $p < 0.05$, ***: $p < 0.001$ ($n = 4$).

Collagen Synthesis

Collagen synthesis was inhibited 44% for 1 day, 31% for 3 days and 53% for 7 days at 22 mM concentration of glucose (Fig. 3). High mannitol (16.5 mM plus 5.5 mM glucose), an

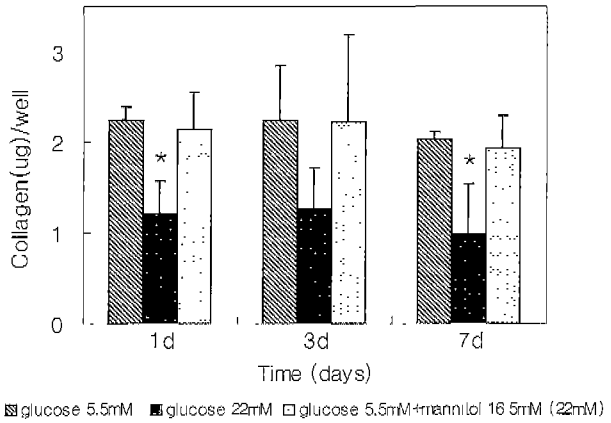

Fig. 3. Collagen synthesis in high glucose concentration (22 mM) on UMR106 cells. Cells were cultured in 5.5 mM glucose, 22 mM glucose and 5.5 mM glucose + 16.5 mM mannitol for 1 day, 3 days and 7 days. The medium was replaced with the same fresh medium on 3 days. Data are shown as the mean ± S.D. *: $p < 0.05$ ($n = 4$).

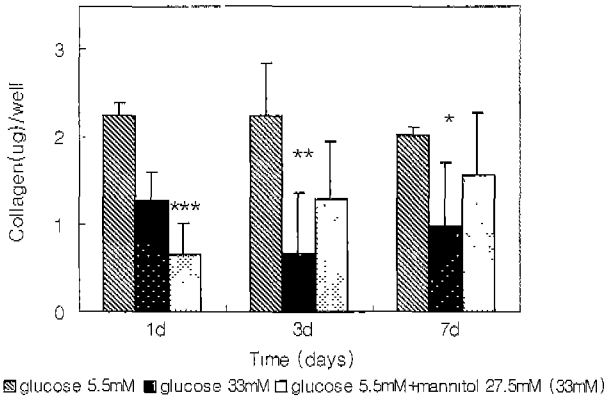

Fig. 4. Collagen synthesis in high glucose concentration (33 mM) on UMR106 cells. Cells were cultured in 5.5 mM glucose, 33 mM glucose and 5.5 mM glucose + 27.5 mM mannitol for 1 day, 3 days and 7 days. The medium was replaced with the same fresh medium on 3 days. Data are shown as the mean ± S.D. *: $p < 0.05$, **: $p < 0.01$ ($n = 4$).

osmotic control for high glucose, did not affect the collagen synthesis, supporting the specificity of this action of glucose in UMR106 cells (Fig. 3). In the 33 mM concentration of glucose, collagen synthesis was inhibited 41%, 63% and 53% for 1 day, 3 days and 7 days, respectively (Fig. 4). Mannitol treatment (27.5 mM plus 5.5 mM glucose) also showed a little inhibition on the collagen synthesis in 3 day and 7 days, whereas it significantly inhibited collagen synthesis in 1 day (Fig. 4).

Protein Assay

In the protein assay with high glucose concentration, it appeared to induce significant reduction of protein levels. The results revealed more reduction in 33 mM than 22 mM concentration of glucose (Fig. 5).

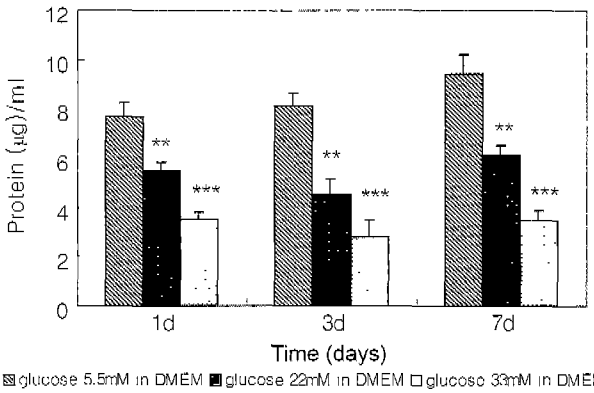

Fig. 5. Protein assay in high glucose concentration on UMR106 cells. Cells were cultured in 5.5 mM glucose, 22 mM glucose and 33 mM glucose for 1 day, 3 days and 7 days. The medium was replaced with the same fresh medium on 3 days. Data are shown as the mean ± SD. **: $p < 0.01$, ***: $p < 0.001$ ($n = 5$).

DISCUSSION

Diabetes is marked with high glucose contents and is further associated with bone loss (McNair *et al* 1979, Levin *et al* 1976), resulting the decrease in bone forming cells osteoblast, and decreased osteoid. Such fact provides logic for altered osteoblast growth and function. The possible mechanism of such action of glucose is presumably due to the fact that these cells are sensitive to glucose supply (Williams *et al* 1997, Terada *et al* 1998, Balint *et al* 2001). An apparent decrease in bone mass turnover observed with high glucose is due to decreased clearance or metabolism of bone markers such as alkaline phosphatase and collagen synthesis activities that are basics of the scale of bone formation.

A phenomenon of decrease in alkaline phosphatase activity with exposure of UMR106 cells to high glucose concentration of 22 mM to 33 mM suggests great inhibitory influence of high glucose on bone formation. We also observed the increased inhibition with prolongation period from 1 to 3 days both at 22 mM and 33 mM glucose concentration. At a concentration of 22 mM inhibition for day 1 was 38%, which increased to 46% on days 3, but somehow got saturated at 47% on day 7. In relation to higher glucose concentration of 33 mM, the same increase in the inhibition has been observed: which is 61% on day 1 to 66% on day 3 and finally 73% on day 7. Thus high glucose content has inhibitory effect on ALP activity. The higher the glucose concentration, the greater the inhibition. It seems that the rate of inhibition is high at initial period although higher inhibitory trend is maintained but degree of further inhibition on prolongation of days is low. The higher degree of inhibition leads to lower bone formation activity and thus low bone matrix production. The inhibitory action of high glucose on the alkaline phosphatase activity may be partially due to osmotic stress since high mannitol treatment also had some inhibitory effect on the alkaline phosphatase activity. However, it is also possible high glucose itself may contribute to the development of metabolic defects in bone cells.

In response to the other bone formation measuring scale the collagen synthesis, similar inhibition were observed on collagen synthesis; With 22 mM glucose, there is slightly decrease in inhibition for days 3 with further acceleration on days 7. Such trends are not observed with high glucose concentration of 33 mM, where there is a gradual increase in inhibitory from day 1 to day 3; however, it decreased with further prolongation. In prolongation, such situation might occur due to involvement of glucose in some other cellular metabolism; such hypothesis also gets support from little inhibition of 33 mM mannitol concentration.

Such inhibitory observation with high glucose seems to be relatively glucose specific in collagen synthesis as similar concentration with mannitol (22 mM) did not have significance effect while there was a trend of decreasing collagen synthesis with mannitol (33 mM). However in a broader sense, high glucose has the ability to inhibit the markers of bone formation activity. Taken together, high glucose itself has some cellular metabolic effects leading to the inhibition of alkaline phosphatase and collagen synthesis; however, high glucose also could act as a osmotic stresser to induce modulation of bone metabolism. Our findings are in agreement with the similar finding of Silbiger *et al* (1993), where reduced synthesis of collagen in diabetic rats were observed and Terada *et al* (1998) where growth inhibitory effect of bone cells by high glucose has been recorded. Reduced protein levels with high glucose further confirmed the involvement of high glucose in its inhibitory action on bone formation.

There are some opposite findings in terms of high glucose effect on alkaline phosphatase and collagen synthesis: Balint *et al* (2001) suggestged that high glucose (15 mM) treatment for 30 days increases alkaline phosphatase in MC3T3-E1 cells and Zayzafoon *et al* (2000) found that high glucose (22 mM) treatment for 1 day increases collagen gene expression in MC3T3-E1 cells. These discrepancy may be due to difference in cell type, glucose concentration, and/or incubation times. In conclusion, it is clearly evidenced that high glucose inhibits bone formation activities and its mode of action could be due to osmotic stress as well as accumulation of glucose metabolite in the UMR-106 osteoblast cells. Further studies will be necessary to delineate molecular mechanism of high glucose on the bone metabolism.

ACKNOWLEDGEMENT

This study was supported by a grant of the Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (02-PJ1-PG3-20905-0001)

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