Appraisal of fibroblast viability in different concentration of glucose as mimicry diabetic condition

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ABSTRACT

Diabetes mellitus is a common name for a group of diseases of a much diversified etiopathogenesis, characterized by chronically increased concentration of blood glucose. Diabetes results from alterations of various genes, each having a partial and additive effect. The inheritance pattern is thus complex, and environmental factors play an important role in favoring or delaying the expression of the disease. Diabetes can cause devastating complications, including cardiovascular diseases, kidney failure, and blindness, leg and foot amputations, delay in wound healing, which often result in disability and death. Fibroblast cells play a critical role in wound healing. They are the most common cells of connective tissue in animals. Tissue damage stimulates fibrocystic and induces the mitosis of fibroblasts. Wound healing processes in diabetic patients are so longer and sometimes cause to cut damaged tissue.

In this study Fibroblasts were isolated from foreskin and cultured as primary cell culture in different concentrations of glucose (8.8 mmol/l, 13.13 mmol/l, 18.31 mmol/l, 27.7 mmol/l, 37.18 mmol/l, 47.17 mmol/l, 83.24 mmol/l, 124.8 mmol/l and 166.4 mmol/l) for 48h incubation time. Traditionally, the determination of cell growth is done by counting viable cells after staining witha vital dye. Among several approaches have been used in the past, The MTT method of cell determination is most invaluable to cultures which are prepared in multiwall plates. This assay is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells.

The results of this preliminary study suggest that altered glucose concentrations may affect fibroblast behavior in ways that are important for tissue repair and wound healing. The cells had low level of confluency and viability and in high concentration fibroblast had very low cell division.

Keyword: Diabetes, fibroblast, in vitro model, MTT assay, glucose

INTRODUCTION

Diabetes mellitus is a complex of metabolic disorders associated with insufficiency of insulin secretion, insulin action or both, and is manifested by hyperglycemia [1–3]. Diabetes is diagnosed when fasting blood glucose exceeds 6.9 mmol/l, or casual or 2-h glucose in a glucose tolerance test exceeds 11 mmol/l [4]. Control of blood glucose in vertebrate organisms is accomplished essentially by the action of two pancreatic hormones, i.e., insulin and glucagon, with the participation of epinephrine, ACTH, growth hormone and glucocorticoids occurring under special circumstances, such as stress. Insulin is released by islet beta cells in response to an increase of blood glucose (usually after a meal). It suppresses glucose production and stimulates the uptake and storage of glucose in skeletal muscle and liver. It also suppresses lipogenesis in the fat tissue and stimulates amino-acid synthesis in skeletal muscle. During a fasting state, when blood glucose is low or during stress requiring mobilization of energy, insulin secretion is suppressed and glucagon is released into the circulation by pancreatic α cells, opposing the action of insulin to increase the release of stored

energy resources for use by the organism[5,6]. Diabetes can cause devastating complications including car-diovascular diseases, kidney failure, leg and foot amputations, and blindness, which often result in disability and death. Diabetic nephropathy is damage to the kidney because of diabetes; it is a common diabetic complication and a leading cause of death in people with diabetes [7].

Fibroblast, connective tissue is the most abundant cell types that synthesis whole of strings connective tissue and organic matter of basal substance. A protein called collagen is the main constituent of the composition of the extracellular environment [11]. There are different types of this protein that causes we have a healthy or fibrous skin. Fibroblast is a one of the major cells to produce collagen and other extracellular material environment. Therefore, these cells have the most important role in the formation of tissue structure and wound healing [12].

Measuring viability and growth of cells with different methods is done, Accurate method to evaluate the survival of cells normally is yellow tetrazolium salt (MTT assay).The salt absorbed by living cells and cause the formation of purple crystals are insoluble Formazan. These crystals will be solution outside the cell by adding a detergent. This color can be measured by spectroscopic methods and measurement. There is a linear relation between the number of viable cells and absorption for each cell. This relationship allows providing accurate determination of any changes in the rate of cell proliferation. In this study, MTT assay method was used [9].

MATERIAL AND METHODS

Skin fibroblast cultures:

Fibroblast of foreskin (Skin Research Center of Shohada Hospital, Tehran) was isolated from human by collagenase digestion as previously described [8, 10].Early fibroblast were cultured overnight in DMEM containing 2 mM L-glutamine, 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 ° C under an atmosphere of 95% air and 5% CO2 prior to experimentation [10].

Method of assessing the vital activity of different concentrations of glucose and toxicity:

MTT method based on restoring color (dimethylthiazol diphenyl tetrazolium bromide) to an insoluble product (Formazan) violet blue by mitochondrial redoctase enzyme activity in living cells. Fibroblast cells after incubation in 48 hour with different concentrations of glucose, included (8.8 mmol/l, 13.13 mmol/l, 18.31 mmol/l, 27.7 mmol/l, 37.18 mmol/l, 47.17 mmol/l, 83.24 mmol/l, 124.8 mmol/l and 166.4 mmol/l), Plates that the temperature 37 ° C and CO2 %5 were incubated and MTT solution 0/5 mg/ml were added to plates and again they were incubated for 3 to 5 hours .After this time, cell supernatant was removed and instead of that the solution 200 micro liter DMSO (Merck, Germany) was added to the corresponding holes. Then the plate was read by a plate reader at 570 nm [9].

RESULTS

Fibroblast cells were captured by inverted microscope respectively, before and after adding different concentrations of glucose. In this study, the mechanism of action of different glucose concentrations on human fibroblast cell line in vitro was examined and also fibroblast cell line as positive control was used. The relevant results are shown in the following figures. The cell proliferation and morphological changes were obvious in different concentrations of glucose, (8.8 mmol/l, 13.13 mmol/l, 18.31 mmol/l, 27.7 mmol/l, 37.18 mmol/l, 47.17 mmol/l, 83.24 mmol/l, 124.8 mmol/l and 166.4 mmol/l), figure 1.

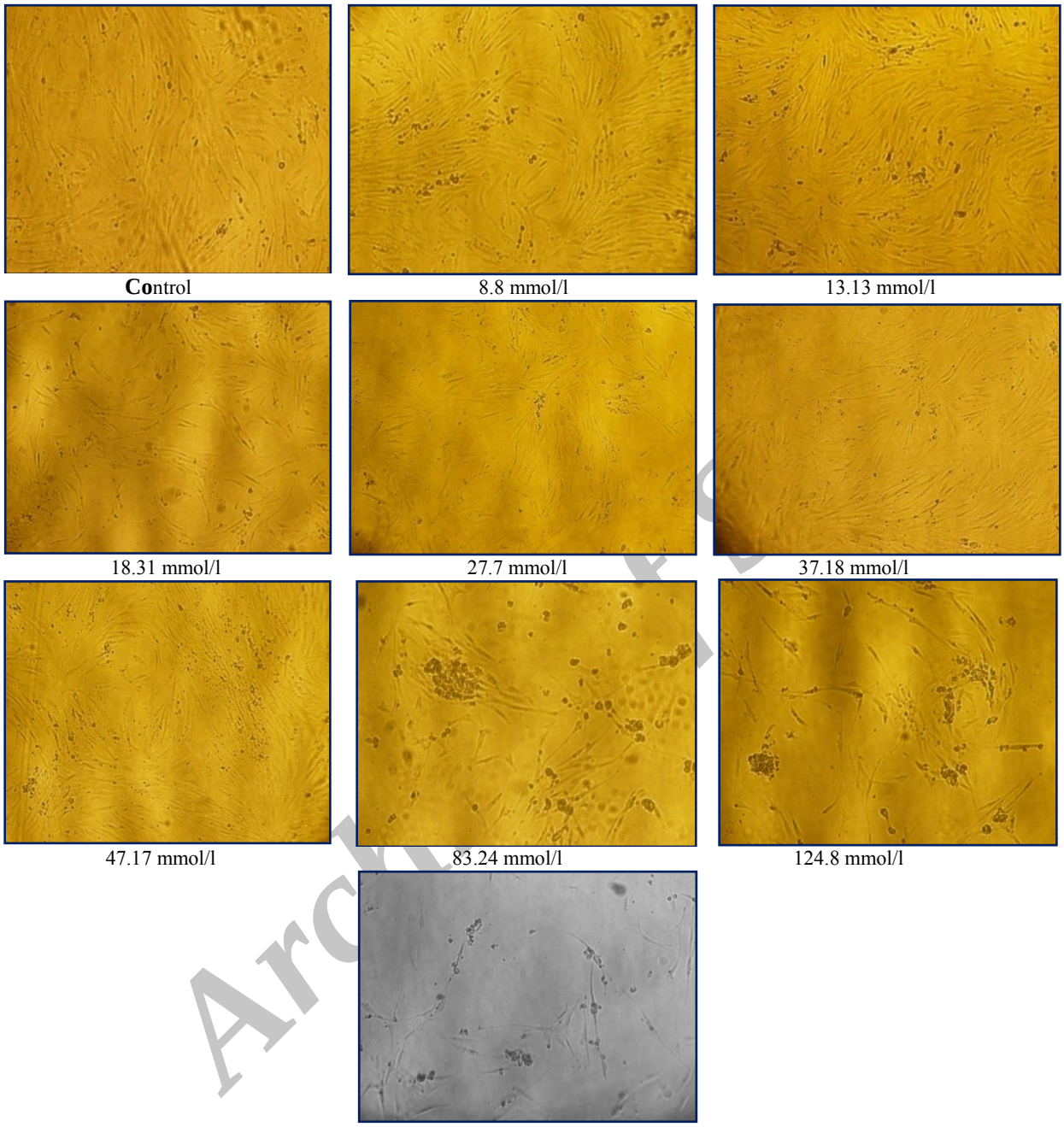

Figure 1: treatment of fibroblast in different concentration of glucose

Morphology of cells after incubation time of 48 hours has changed in the presence of different concentrations of glucose. Furthermore, the number of cells is severely reduced and in some cells, the granulations were observed. According to (Chart 1) the effect of glucose concentration shows can be seen that the glucose in the incubation period of 48 hours to concentrations of 18.31 mmol/l inhibitory effects of certain did not show while at the concentration's greater effect of inhibition has been observed. Moreover, in the concentration's of 83.24 mmol/l significant decline in proliferation, and cell morphology changes also were shown a clearly.

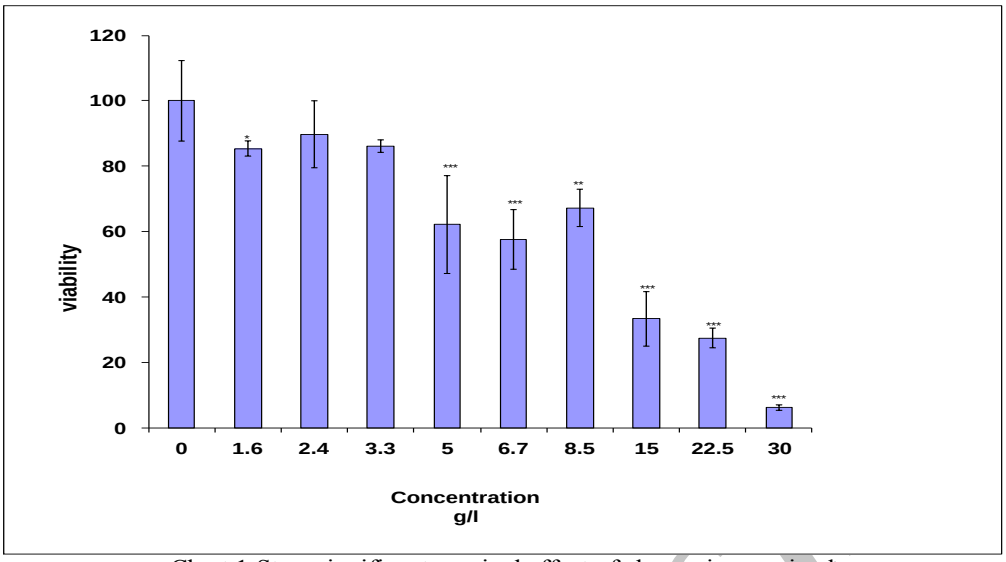

Chart 1:Stars significant survival effect of change is examined the values *: P <0.05, **: P <0.01, ***: P <0.001 are shown.

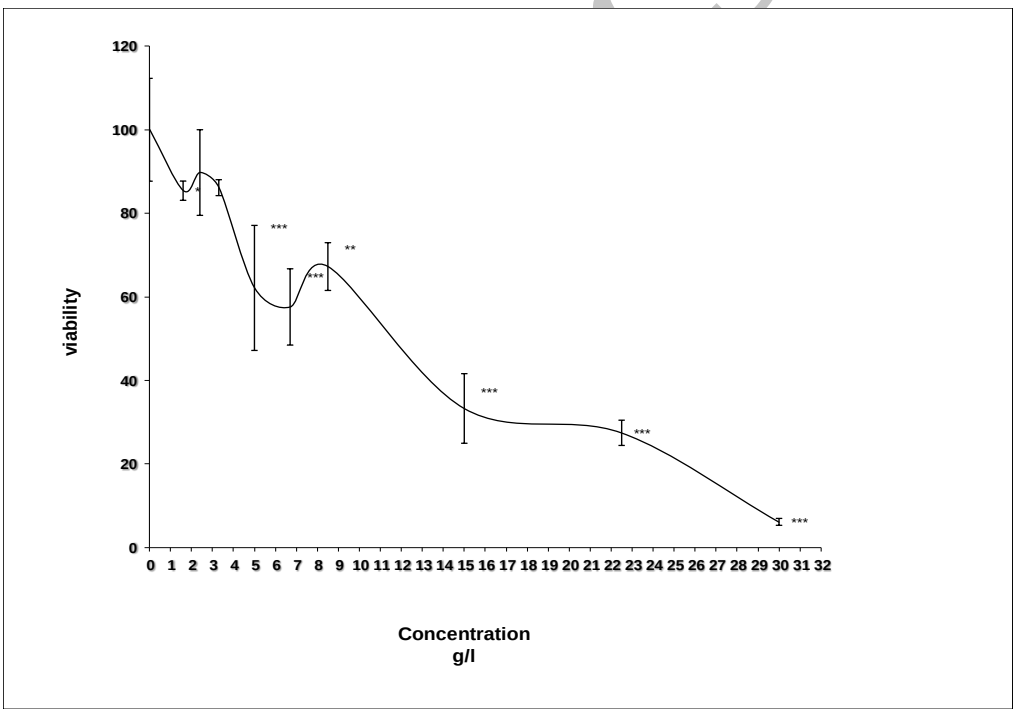

Chart 2: Graph linear effect of glucose concentration changes on the survival fibroblast cells.

DISCUSSION

Diabetes defines as the extra-glucose effects on human cells and this is the result of loss or insufficiency of the insulin secretion in the human body. The high glucose concentration around the cells can give rise to the metabolic changes within the cells. These changes can be evaluated under in vivo and in vitro conditions. With in vitro model of diabetes, the wide range of changes can be applied for the cell sample, and also all the metabolic changes can be assessed by changing cell conditions. For providing an in vitro diabetes model, the normal amount of glucose on the cell surface can be evaluated. The above mentioned amount is 5mmol/l.

With regard to the fact that the diabetes side effects will appear during a long time, the occurrence of the side effects can be attributed to the high-glucose concentration at a long time. The toxicity effect of glucose on normal fibroblast cells was analyzed according to MTT method. Considering the high concentration of glucose is causing danger, Dose rate higher than normal were applied in the culture mediums. As can be seen in figure 1, one of the factors is stress and this implies that the high doses of glucose have caused the inhibition of cell growth in the medium.

The qualitative changes resulting from applying stress on glucose in the medium is perceptible, but quantitative analyses require a cell count testing and maintenance curve. During MTT cell survival as a function of glucose concentration has been studied. Results indicated cytotoxic effects of glucose are at higher doses than normal on cell's survival. Statistical analyze indicates that 83.24 mmol/l of glucose considerably threatens the cell maintenance and inhibits cell growth. The higher concentration definitely shows 5%-10% decrease in the cell growth as in 166.4 mmol/l concentration, the cell growth has been inhibited, and this leads to cell death. Desired changes in the charts (1-2) shows that glucose concentrations linearly uniform are not inhibited cell growth. For example, 8.8-18.3 mmol/l glucose concentration does not inhibit the cell maintenance, but the higher dose gives rise to the inhibition of cell growth. It seems that a definite concentration of glucose (8.8 mmol/l) causes some changes in fibroblast's vital pathways. These changes are suitable for inhibiting the cell growth.

Considering the higher concentrations, the inhibitory effect of glucose is omitted because the cell maintenance has not decreased rapidly and equally. All the evidence pointed to the conclusion that the other mechanism is activated with higher concentrations, which supports the cell maintenance. This process is stronger than providing the conditions in order to inhibit the cell growth because it can stop inhibitory mechanism for cell growth. With regard to the inhibition of cell growth in concentration higher than 18.31 mmol/l, it seems that the cell pathway is activated, which is done harmoniously with the mechanism of cell growth inhibition towards threatening cell maintenance.

Thus it is reasonable that the effect of glucose on only one receptor and one enzyme does not apply. Expected effect on a set of genes and cells are effective. Therefore, study and evaluation of diabetes from in vitro condition express of this issue that there are multiple and complex mechanisms that it can change normal cell condition to critical condition diabetic. The morphological and vital changes applied for the cells are resulting from the changes, which had been used for proteins and genes expression and finally for the cells involved in this procedure.

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