Hyperglycemia amplifies the osteogenic responses in vascular cells in the presence of elastin degradation products and TGF-β1

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Introduction

Diabetes Mellitus (DM) is a chronic disease in which the body either does not use or produce the glucose metabolizing hormone- insulin efficiently. Currently around 35 million people are affected by diabetes in the United States alone.[1] Calcification of elastin in the arteries of diabetics is a major predictor of cardiovascular diseases. It has been previously shown that elastin degradation products work synergistically with TGF-β1 to induce osteogenesis in vascular smooth muscle cells [2]. **We hypothesize** that high concentration of glucose coupled with elastin degradation products and TGF-β1 (a cytokine commonly associated with diabetes) will cause a greater degree of osteogenesis compared to normal vascular cells. Thus the goal of this study was to analyze the effects of high concentration of glucose, elastin peptides, TGFβ-1 on bone specific markers like Alkaline phosphatase (ALP), osteocalcin (OCN), Core binding factor alpha-1 (CBFA-1). We also hypothesize that elastin degradation products in presence of high glucose cause the increase in expression of the specific elastin laminin receptors (ELR) present on the surface of the vascular cells.

Materials and Methods

Rat aortic smooth muscle cells (RASMCs) were cultured for 3 and 7 days in low glucose (5mM) and high glucose (25mM) DMEM supplemented with 100µg/ml elastin peptides and 10ng/ml TGFβ-1.[2] The cell lysates and conditioned media was examined for matrix metalloproteases-9 (MMP-9) in duplicates by gelatin zymography (clear bands indicate the presence of active MMP-9). Total RNA was extracted from cells with Qiagen's RNeasy Mini Kit and quality and quantity evaluated on an Agilent 2100 Bioanalyzer. Expression of CBFA-1, OCN, ALP and ELR genes was analyzed by Real-time PCR using the SYBR green kit (Qiagen), in a Rotorgene 3000 thermal cycler (Corbett Research). Relative gene expression was normalized to ß-2 microglobulin as a housekeeping gene and compared to cells incubated in culture media alone, using the $2^{-\Delta\Delta C}{}_{T}$ method.

Results

When compared to cells in low glucose DMEM, vascular cells showed greater degrees of osteogenesis when exposed to high glucose, elastin peptides, and TGFβ-1. This is apparent from the over-expression of ALP, OCN, CBFA1 genes (Fig.1). Protein data for ALP also corroborated with gene expression. (data not shown). In addition there was also an increased induction of ELR gene suggesting that high glucose concentrations can cause greater ELR expression which has shown to increase osteogenesis [2]. Cellular extracts from cells grown in high glucose showed higher proMMP-9 (Fig.2) whereas conditioned media of cells in high glucose show greater active form of MMP-9 (Fig.3).

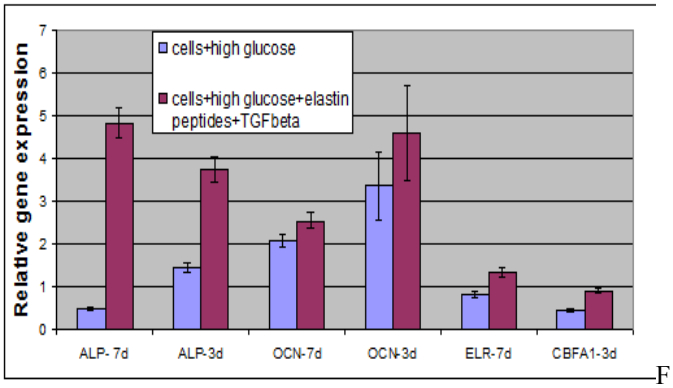

Figure 1: Greater relative gene expression of typical bone markers by vascular cells grown in high glucose, elastin, TGFβ

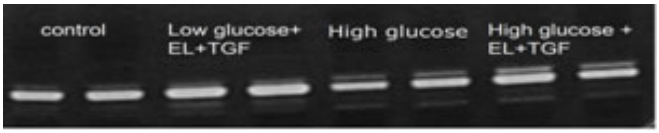

Figure 2: MMP-9 activity in smooth muscle cells (7days)

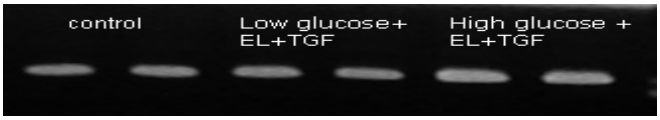

Figure 3: MMP-9 activity in conditioned media (7days)

Conclusions

Glucose coupled with elastin peptides and TGF-β increased ELR expression on RASMCs, which in turn lead to increase in the degree of osteogenesis in vascular smooth muscle cells. In addition, it appears that pro-MMP-9 is produced more by the cells grown in high glucose which is substantiated by the higher MMP-9 activity in the conditioned media of cells in high glucose. Thus Diabetic conditions along with elastin degradation products may make vasculature more prone to calcification.

References

[1] J. H. Martin, S. Mangiafico, and D. J. Kelly, "Role of Statins in Diabetes Complications," *Current Diabetes Reviews,* vol. 5, pp. 165-170, 2009.

[2] A. Simionescu, K. Philips, and N. Vyavahare, "Elastin-derived peptides and TGF-[beta]1 induce osteogenic responses in smooth muscle cells," *BBRC,* 2005.