Effect of undercarboxylated osteocalcin on experimentally induced diabetic cardiomyopathy in male rats and its possible mechanisms of action.

Thesis

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صدق الله العظيم,,

"وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا"

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Abstract

Backround: Diabetes mellitus (DM) is a major epidemic of this century. Epidemiological studies indicate that more than 70% of diabetes mellitus (DM) patients die of cardiovascular disease (CVD). Damage to the diabetic myocardium in the absence of hypertension and coronary artery is termed diabetic cardiomyopathy (DCM). Osteocalcin (OC) is a bone matrix protein that undergoes post translational carboxylation at 3 sites by carboxylase enzyme. Full carboxylation is essential for retaining OC in bone bound to hydroxyapatite. The undercarboxylated (ucOC) form is released in the blood as a hormone with several actions on energy and glucose metabolism. It stimulates insulin secretion and enhances insulin sensitivity at least in part via stimulating adiponectin. Osteocalcin was found to be related to lower cardiovascular risk in human adults. Use of vitamin K antagonists (eg. Warfarin) can block the activity of carboxylase enzyme, increasing the undercarboxylated portion of OC. The present work was designed to study the possible prophylactic and therapeutic effects of elevated plasma ucOC on diabetic cardiomyopathy in type II DM and associated risk factors, and to investigate the role of possible underlying mechanisms. The possibility of a direct action for osteocalcin on the heart was also studied.

Methods: 56 adult male albino rats were divided into 6 groups (n=8/group): control group (further divided to control A and B), diabetic group, diabetic warfarin prophylaxis group, DCM untreated group, DCM warfarin treated group and normal warfarin treated group, for these groups, isolated heart function was assessed by measuring LV developed pressure, $\Delta P/\Delta T$ and - $\Delta P/\Delta T$ using powerlab data acquisition system aided by Langendorff heart perfusion apparatus. Blood samples were collected for measuring Fasting blood glucose and serum insulin as well as glycosylated hemoglobin and HOMA IR was calculated, besides, lipid profile was investigated by measuring serum triglycerides, VLDL and HDL for evaluation of risk factors in addition to serum ucOC and adiponectin. Cardiac tissue samples were taken to investigate malonaldehyde (MDA) content, acyl co-A dehydrogenase gene expression, TNF α, Bax/BCL2 ratio, VEGF gene expression and

SERCA gene expression. Besides, the gene expression of OC receptor (GPRC6A) was assessed in the cardiac tissue.

Results: According to the results of the current study, ucOC elevation via antagonizing the carboxylase enzyme using warfarin was accompanied by increased serum adiponectin and upregulated myocardial OC receptor in addition to favorable metabolic effects enhancing insulin secretion, lowering blood glucose and improving insulin sensitivity and lipid profile. Increased ucOC showed a protective effect against the development of DCM when administered as prophylaxix, and although not fully reversed, the condition of the myocardium was significantly improved when ucOC was elevated even after the settlement of DCM as a treatment. These favorable effects of ucOC were proved by functional assessment of isolated heart, which showed improvement in LV developed pressure systolic $\Delta P/\Delta T$, and diastolic - $\Delta P/\Delta T$. Further investigating the underlying mechanisms emphasized that effect, showing decreased cardiac MDA content, acyl co-A dehydrogenase gene expression, TNF α and Bax/BCL2 ratio, together with increased VEGF gene expression and SERCA gene expression.

Conclusions: The present study added an evidence for the protective effect of ucOC against diabetic drawbacks through improving plasma glucose level, insulin secretion and sensitivity as well as ameliorating dyslipidemia. These metabolic benefits are considered through both direct action of ucOC on the pancreas, and indirectly through adiponectin. ucOC was further proved to have a beneficial effect on diabetic heart, protecting it from the development DCM and even improving the state of the heart that has already developed DCM. This might be related to the ucOC systemic effects or to local effects through antagonizing the oxidative stress, anti apoptotic and/or anti inflammatory factors. The associated upregulated cardiac OC receptor expression raised the possibility of a direct action of ucOC on diabetic heart, a point that needs further studies. Applicability in humans also needs further study.

Key words: DCM, Warfarin, ucOC, Adiponectin, MDA, BAX/BCL2, SERCA.

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