Ghrelin versus stem cells as cardio-protective approaches and their synergistic effect in experimentally - induced myocardial injury in rats

Thesis

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SUMMARY

Cardiovascular diseases (CVD) remain one of the most serious health issues, accounting for substantial morbidity and mortality throughout the world. Cardiac injury occurs due to myocyte necrosis, apoptosis, increased membrane permeability and release of proteolytic troponin degradation products. Myocardial injury may occur in response to myocardial ischemia or drug induced or multifactorial etiology as in kidney injury and pulmonary embolism.

Ghrelin hormone has been recently discovered to show cardioprotective effects via its anti-inflammation, anti-apoptosis effects and through inhibiting sympathetic nerve activation and endothelial dysfunction. MSCs have the ability to home inside the damaged or the ischemic tissues in which they secrete growth factors, angiogenic, anti-apoptotic factors and regenerative molecules that can promote tissue regeneration. **The aim of the present study** was to investigate the possible protective role of ghrelin in comparison to Wharton Jelly derived Mesenchymal Stem in Doxorubicin- induced myocardial injury in male albino rats with highlighting the possible underlying mechanisms. The study aims to investigate in addition their possible synergistic effect.

Forty male Wistar albino rats were included in this study divided into five groups (8 rats/group):

Group (I): Control normal: Healthy rats treated with single intra-peritoneal (i.p) injection of normal saline 1 ml/kg.

Group (II) Myocardial injury (MI): MI was induced by single i. p injection of doxorubicin (DOX) at a dose of 25mg/kg given once which is well proved to produce cardiotoxic effects.

Group (III) MI treated with ghrelin: Rats were injected with ghrelin at a dose of (150 µg/kg subcutaneously) one day before induction of MI that was induced as in group II.

Group (IV) MI treated with mesenchymal stem cells MSCs: Rats were injected intravenously through the tail vein with labeled MSCs (1×10^6 in 0.5 ml saline) three days before DOX injection. MI was induced as in group II.

Group (V) MI treated with both Ghrelin and MSCs: Both MSCs then ghrelin were administered; three days and one day before DOX injection respectively with the previously mentioned doses. MI was induced as in group II.

At the end of the experiment and after assessing the cardiac performance parameters (EF, FS, LVDP, dP/dT) and HR, both blood & tissue samples were collected from all animals. Among the five studied groups, plasma levels of cardiac enzymes (Ck-mb, LDH, c-Troponin) and oxidative biomarkers in both plasma and cardiac tissue (MDA, Catalase, SOD) were measured. Moreover, the protein blotting of the apoptotic biomarkers (BCL2, P53 and Cleaved Caspase 3) was detected in the cardiac tissue. Both histopathological examination of the cardiac tissue using H&E and Masson stains and immunohistochemical detection of Nrf2 and HO-1 were also performed.

The results revealed a significant increase in plasma cardiac enzymes, both plasma & cardiac MDA, cardiac P53, cleaved caspase 3 levels and HR, meanwhile a significant decrease noticed in both plasma & cardiac catalase, SOD, cardiac BCL2 levels, EF, FS, LVDP and dP/dT in myocardial injury group compared to the healthy control rats **as DOX promote fibrosis through excessive fibroblast accumulation and scar formation that leads to increase cardiac stiffness and decrease contractility**. Myocardial injury treated groups either with ghrelin alone, MSCs alone or combined therapy showed a significant increase in both plasma &cardiac catalase, SOD, cardiac BCL2, EF,FS, LVDP, dP/dT levels, meanwhile the therapy resulted in a significant decrease in ck-mb, LDH, c-troponin, both plasma &cardiac MDA, cardiac P53, cleaved caspase 3 and HR compared to the non-treated myocardial injury group. As stem cells can differentiate and behave mechanically and electrically as innate cardiomyocytes.

The solo treatment groups either with ghrelin or stem cells revealed insignificant difference in most of the parameters compared to the normal control

group with the exception of a noticed significant difference in cardiac apoptotic markers and both plasma & cardiac MDA in ghrelin treated & MSCs treated groups compared to the normal values in control group. Another significant difference was also noticed in MSCs treated group regarding both plasma & cardiac MDA, cardiac catalase, plasma SOD and HR when compared to the control group. The protective impact of ghrelin against cardiac injury was explained through inhibition of cardiac IL-6 production, which in turn activates JAK2/STAT3 signaling in the heart.

As regarding the cardiac enzymes & oxidative markers, the cardiac performance parameters and HR there was no significant change in the combined treatment group compared to the other treatment groups. Meanwhile a significant difference was noticed in the cardiac apoptotic markers in the combined treatment group in comparison to the solo treatment groups indicating the superiority of the combined therapy above the effect of either ghrelin or stem cells alone in alleviating the apoptotic process. The combination therapy in group V has no more to do than the solo treated groups because it has been noticed from the prior data that the solo treatment, whether by ghrelin alone or stem cell alone, improved the majority of the parameters close to the normal levels.

According to the histopathological examination with H&E in the myocardial injured group there was disruption of cardiac muscle architecture, wavy myocardial fibers and pyknotic peripherally situated nuclei, congested blood vessels and inflammatory cellular infiltrations. The improvement in histopathological features was noticed in ghrelin treated group that showed more centrally located nuclei and few congested blood vessels. In both stem cell & combined treated groups there were orderly arranged myocardial fibers with normal striations, central vesicular nuclei, and absence of both inflammatory cells infiltration and vacuolar degeneration and minimal vascular congestion.

The significant increase in area percent of collagen deposition detected by Masson trichrome staining in MI group compared to other groups was improved by treatment with either ghrelin, MSCs or both. Yet a significant difference still noticed between the three treatment groups and the control group with no significant difference between MSCs & combined treatment groups.