Effect of ghrelin in two models of angiogenesis matrigel plug and hindlimb ischemia In obese and normal male mice

Abstract:

Background: Nowadays, obesity and associated diseases is considered as a serious threat for human societies because rapid increase in worldwide. Adipose tissue for its abilities in rapid and dynamic expansion and shrinkage in periods of excess or demand of energy exposure is illustrated as a unique plastic tissue that angiogenesis and vascular system have a crucial role in plasticity. Ghrelin is a peptide hormone mainly derived from stomach in addition, its effects in food intake, regulation of appetite and energy homeostasis, in recent years has been demonstrated as a cardiovascular hormone for many cardiovascular effects. The objective of present study was to evaluate the effect of ghrelin on angiogenesis in matrigel plug and hindlimb ischemia models in obese and control male mice.

Materials and methods:

64 male C57BL/6 mice with an approximately weight of 15-20 g were randomly assigned two groups of obese and control. For induction of obesity, obese group consumed HFD (labratories BioServ, Cat #F3282, USA) included 59% fat, 27% carbohydrate, 14% protein for 14 weeks. Then each group were subdivided into 4 groups include: 1- Growth factor- reduced matrigel plug (BD Biosciences; 600 µl) containing bFGF (basic FGF) (Sigma-Aldrich, St.Louis, MO, USA; 100 ng) subcutaneously was injected in the midventral abdominal region of each mice 2- growth factor-reduced matrigel plug (BD Biosciences; 600 µl) containing bFGF (basic FGF) (Sigma-Aldrich, St.Louis, MO, USA; 100 ng) with ghrelin (Tocris Co. Bristol, UK; 100 µg/Kg) was injected in the midventral abdominal region of each mice 3- The mice underwent unilateral hindlimb surgery and received ghrelin

solvent 4- The mice underwent unilateral hindlimb surgery and Ghrelin was injected subcutaneously, twice daily, at the dose of 100 µg/kg for each mice 10 days.

After 10 days animals were weighted, determined blood glucose by glucometer and sacrificed by cervical dislocation. Blood samples were taken for measurement of serum levels of VEGF ¸ sVEGFR1 ¸ NO ¸ leptin ¸IL-6¸ hsCRP and insulin. Also matrigel plug, the skeletal muscle and heart were harvested and fixed in formalin 10% for capillary density analysis. In addition, epididymal fat was removed for adipocyte cell number and size study and thoracic aorta for fatty streak formation.

Results:

In this study was no significant difference in the cap / fiber ratio between obese and control groups ($p>0.05$) However, HFD increased apoptosis in the ischemic skeletal muscle ($p<0.05$). ghrelin treatment did not alter angiogenesis that was expressed cap /fiber ratio and apoptosis in the skeletal muscle ($p>0.05$).

HFD significantly increased angiogenesis that was expressed (the number of CD31 positive cells) in matrigel plug and the heart than standard diet ($p<0.05$) however, ghrelin did not change angiogenesis in the matrigel plug and heart in obese and control group (($p>0.05$).

HFD significantly increased serum level of leptin, insulin and NO ($p<0.05$) whereas did not affect serum level of VEGF and sVEGFR1 ($p>0.05$). Ghrelin treatment increased serum level of VEGF and reduced leptin and NO levels in obese animals ($p<0.05$).

In obese group adipocyte cell number significantly decreased than to control group while adipocyte cell size increased ($p<0.05$). Fatty streak formation

increased in obese group than control animals ($p<0.05$) and ghrelin administration did not change adipocyte characteristic and fatty streak formation in obese and control groups ($p>0.05$).

Conclusions:

Although systemic administration of ghrelin reduced serum leptin, NO and elevated VEGF levels in obese mice however, it could not alter angiogenesis in the skeletal muscle, matrigel plug and heart. Of course, the exact effect of ghrelin on angiogenesis needs further confirmation.

Keywords: Obesity, Angiogenesis, Ghrelin, Matrigel plug, Hindlimb ischemia