Pharmacological characterization of the ghrelin receptor mediating its protective effect against oxidative stress in osteoblast-like MC3T3-E1 cells.

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Age-related bone loss and resultant osteoporosis is a multifaceted and multifactorial disease. It involves progressive loss of both bone quantity and quality due increases in bone resorption and decreases in bone formation. A number of genetic, hormonal, and biochemical players have been implicated in this process. Recently, the development of osteoporosis has been associated with increased levels of oxidative stress in osteoblasts, suggesting that this may be critical component of the pathophysiology of bone loss. Thus antioxidants could be of interest as potential candidates for osteoporosis treatment.
Different compounds such as flavonoids, N-acetyl cysteine and vitamin E, have been reported to protect osteoblastic function from oxidative stress. Recently, the attention has focussed on ghrelin (AG) the endogenous ligand for the growth hormone secretagogue receptor 1a isoform (GHS-R1a), which exhibits antioxidant effects in many organs such as heart, pancreas and lung and stimulates osteoblast proliferation and differentiation.
The aim of the present study was to investigate the effect of AG on osteoblast viability after exposure to tert-butylhydroperoxide (t-BOOH) a chemical compound commonly used to induce oxidative stress in biological systems.
We used, MC3T3-E1 (osteoblast precursor cell line derived from Mus musculus (mouse) calvaria) were exposed to t-BOOH (250μM) for 150 minutes with or without pre-treatment (120 min before) with AG different concentractions (10^{-5} – 10^{-11} M). Cell viability was analyzed using 3-(4,5-dimethyl-2-thiazoly)-2,5-diphenyl-2h-tetrazolium bromide assay (MTT assay).
t-BOOH treatment significantly decreased MC3T3-E1 viability whereas, AG (10^{-9} M) significantly prevented the cytotoxic effect of t-BOOH treatment.
We then examined whether GHSR1a is involved in the protective effect of AG against t-BOOH-induced oxidative stress. For this purpose, we used the selective GHS-R1a agonist, EP1572 and a non-acylated form of ghrelin (DAG), which does not bind to GHS-R-1a.
EP1572 (10^{-7} – 10^{-11} M) has no effect against t-BOOH-induced oxidative stress. DAG (10^{-5} – 10^{-11} M) exerts a strong protective effect against oxidative stress induced by t-BOOH reaching a maximum effect at 10^{-10} M, indicating that GHSR-1a is not involved in the antioxidant activity of ghrelin. This assumption is supported by the results obtained with the GHSR-1a antagonist, D-Lys GHRP6 (10^{-7} M) which did not prevent the protective effect of AG.
In conclusion, we have shown that both AG and DAG protect MC3T3-E1 against an oxidative insult, and that a ghrelin receptor different form 1a is involved in this activity.
Future investigations will be necessary to determine the molecular mechanisms as well as the in vivo relevance of our findings. Overall, our data suggest that ghrelin could be considered a promising compound for oxidative stress-related bone injury.