Type of presentation: Poster

LS-14-P-2348 The effects of ghrelin against tunicamycin-triggered endoplasmic reticulum stress in C6 glioma cells

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Impaired function of the endoplasmic reticulum (ER) leads to accumulation of misfolded proteins and induction of the unfolded protein response (1). The aberrant accumulation of specific misfolded proteins in intracellular inclusions or extracellular aggregates has been recognized to be crucial in the pathogenesis of some neurodegenerative diseases (2). Recent evidences suggest that astrocytes are important players in neurodegenerative disorders (3). We used an in-vitro ER stress model induced by tunicamycin (T) in C6 glioma cells which is derived from rat astrocytes. Ghrelin (G) is a natural peptide ligand of the growth hormone secretagogue receptor which is known to stimulate growth hormone secretion (4). The aim of this study was to investigate the potential protective effects of G on ER stress model.

Cells were cultured in DMEM-F12 containing 10% FBS at 37˚C with 5% CO2 in a humidified incubator. Dose and time dependent preliminary experiments were performed by 0.5, 1, 2.5, 5 and 10 µg/ml T for 2, 6, 12 and 24 hours (h). 2.5 µg/ml dosage of T was chosen and applied for 12 and 24 h. Cells were divided into four groups; control group, T mediated ER-stress group, G (100nM) treated group and G+T group (G administered one hour before T application). At the end of incubations, cells were fixed and immunocytochemical analysis was performed by using ER stress marker anti-GRP78/BiP, and BrdU antibodies. Semi-quantitative analysis of immunoreactions and proliferation percentages were statistically evaluated with ANOVA using GraphPad InStat DTCG software.

BrdU assay showed that G increased the proliferation level of cells significantly according to control group (p<0.05) while 2.5 µg/ml T decreased the level significantly (p<0.01) (Fig 1,2). When G was applied to cells before T, a significant increase in proliferation level was observed according to T group (p<0.01) (Fig 1,2). Statistical analysis of immunoreactions against GRP78/BiP showed that 2.5 µg/ml T for 12 and 24 h extremely increased the expression level of BiP significantly compared to control group (p<0.05); however, G substantially suppressed the effect of T when applied before T (p<0.01) (Fig 3,4).

According to our results, G has a protective effect on neuroglial cell proliferation suppressed by ER stress. It also affects the expression of GRP78/BiP, thus equilibrating between cell surviving and apoptotic cell death. Further research needs to be done for new approaches to applications of ghrelin as a neuroprotective agent.

References

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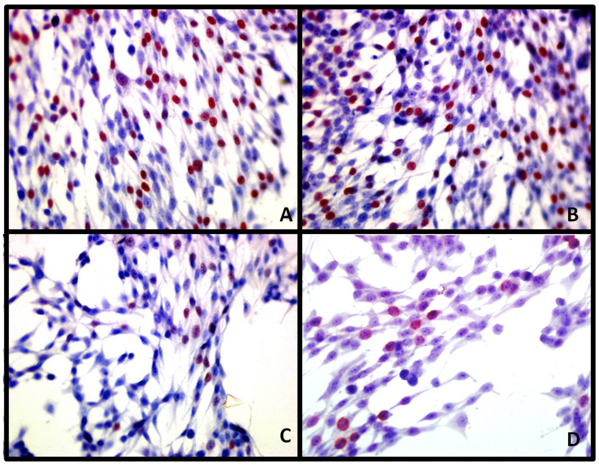

Fig. 1: BrdU immunoreactions in C6 glioma cells. Control group (A), Ghrelin group (B), Tunicamycin group (C), Ghrelin+Tunicamycin group (D). Hematoxylin counterstain, 40X.

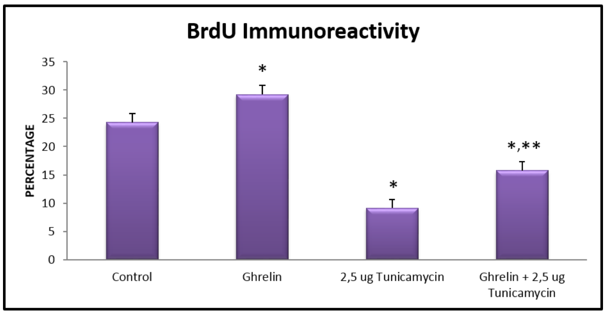

Fig. 2: Percentage analysis of distribution of BrdU expression in C6 glioma cells.*P<0.05; compared to Control group, **P<0.01; compared to Tunicamycin and Ghrelin groups.

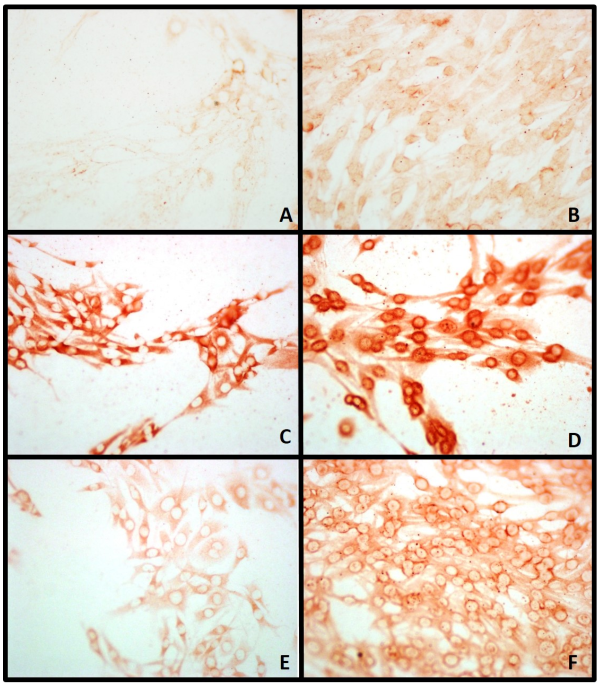

Fig. 3: GRP78/BiP immunoreactions in C6 glioma cells incubated for 12 (left side) and 24 (right side) hours. Control group (A,B); Tunicamycin group (C,D); Ghrelin+ Tunicamycin groups (E,F), 40X.

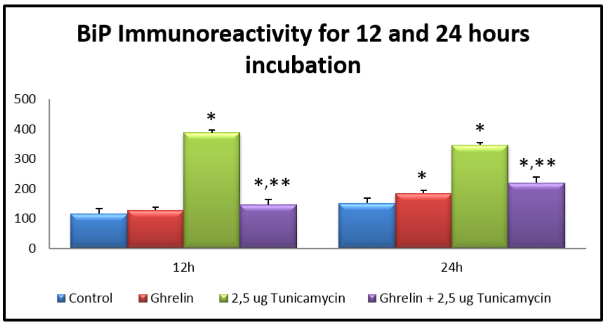

Fig. 4: H-SCORE analysis of intensity of GRP78/BiP expression in C6 glioma cells. *P<0.05; compared to Control group, **P<0.001; compared to Tunicamycin group.