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CO-014 - PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE 2 (PTGS2) CONTRIBUTES TO THE BL001-NR5A2/LRH1 ANTI-APOPTOTIC SIGNALLING PATHWAY IN ISLET BETA CELLS UNDER CYTOKINE ATTACK

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Resumen

Introduction and objectives: We previously reported that BL001, a small chemical agonist of the nuclear liver receptor homolog 1 (LRH1 a.k.a. NR5A2), protects both mouse and human islets against-stress induced apoptosis *in vitro* as well as to revert hyperglycemia in mouse models of type 1 diabetes mellitus. To gain insight into the mode of action of BL001, global gene expression profiling was conducted on mouse islets treated with increasing concentrations of the compound. Surprisingly, most BL001 targeted genes were involved in immunomodulation pathways. Among these genes was the inducible prostaglandin endoperoxide synthase-2 (PTGS2) that was shown to be essential for the resolution phase of the inflammatory process. Herein, we sought to establish whether PTGS2 is a downstream target of the BL001/NR5A2/LRH1 signaling pathway conveying islet beta cell survival under cytokine/inflammation conditions.

Material and methods: Expression levels of PTGS2 were assessed by QT-PCR and Western blot in mouse islets isolated from either WT or conditional beta cell specific LRH1 knock out mice treated or not with BL001 and with cytokines. The RAW264,7 cell line that expresses high levels of PTGS2 subsequent to LPS treatment was used as a positive control. Stomach that expresses low basal levels of PTGS2 was used as a positive control for PTGS2 expression in primary tissue. To assess the contribution of PTGS2 in the BL001-LRH1/NR5A2 anti-apoptotic pathway, the former was silenced using siRNA and islets were then challenged, or not, with a cytokine cocktail and further treated with BL001. Apoptosis was measured through cleaved (Cl.) PARP using either immunofluorescence or Western blotting.

Results: Expression levels of PTGS2 were barely detectable in islets, kidney and untreated RAW264,7 cells as well as stomach that exhibits endogenous expression under basal conditions, whereas both cytokine and LPS treatments time-dependently induced its expression in islets and RAW264,7 cells, respectively. Consistent with our previous results, BL001 induced *ptgs2* expression, an effect that was abrogated in islets lacking LRH1/NR5A2. The combined BL001/cytokine treatment further stimulated *ptgs2* expression above levels detected with either compounds alone. Silencing of PTGS2 resulted in blunted induction of the target in islets treated with cytokines alone or with BL001. More importantly, PTGS2 silenced islets were refractory to the protective effect of BL001 under cytokine attack.

Conclusions: These results indicate that PTGS2 is a key contributor to the anti-apoptotic signaling

pathway of BL001 agonistic activation of NR5A2/LRH1.