EFFECT OF GLP-1-GASTRIN DUAL AGONIST ZP3022 ON PANCREAS GENE EXPRESSION IN ZDF RATS

Jolanta Skarbaliene1, Kristoffer T.G. Rigbøl2, Nils Billestrup2, and Keld Fosgerau1
1Zealand Pharma A/S, Glostrup, Denmark, 2Gubra ApS, Harsholm, Denmark, 3Department of Biomedical Sciences, University of Copenhagen, Denmark

Background and aim
We have previously demonstrated that 8 weeks intervention treatment with ZP3022 markedly improved glycemic control and preserved β-cell function in ZDF rats to a greater extent than currently available GLP-1 agonists.

Here we investigated the effects of short-term (3 weeks) ZP3022 treatment on pancreatic gene expression in ZDF rats.

Materials and Methods
In vivo study
28 ZDF (fa/fa) rats (12 weeks of age) were stratified into 4 treatment groups with matching HbA1c, blood glucose levels and body weights. ZDF rats were dosed s.c. bid for 3 weeks with either vehicle, ZP3022 (40 nmol/kg), exendin-4 (Ex-4; 30 nmol/kg), or a combination of exendin-4 and gastrin-17 (Ex-4+G-17; 450 nmol/kg, 30 + 80 pmol/kg, respectively). Body weight was recorded daily, blood glucose levels were measured after 2 weeks of treatment in the morning before the dosing. At the termination pancreas tissue was sampled and gene expression profiling was performed using microarray analysis (n=6/group).

Results: Identification of differentially expressed genes
Clustering of the samples from group ZP3022 can be observed in heat map demonstrating that this compound has effects on gene expression not observed when giving exendin-4 alone or in combination with gastrin-17. This observation can be seen from the presence of all samples from a group on the same branch of the phylogenetic tree illustrated on top of the heatmap.

Results: Identification of differentially expressed genes
The MAPK signaling pathway was observed as the highest affected pathways regulated by ZP3022 treatment.

Results: Differentially expressed genes encoding for the specific β-cell/endocrine cell markers
The largest number of differentially expressed genes was found between the vehicle group and ZP3022 group with more than 500 genes differentially expressed at significance level p < 0.01 for multiple testing (Figure 3 A). In addition, Venn analysis of the differentially expressed genes (p < 0.01) demonstrated that samples from exendin-4 monotherapy and combination treatment with exendin-4 and gastrin-17 group had a limited number of unique genes (i.e., not altered with other treatments), whereas a large number of unique genes were detected in ZP3022 group (Figure 3 B).

Results: Body weight and blood glucose levels
Vehicle ZP3022 Ex-4 Ex-4+G-17 4 5 6 7 **
Datasheet for gene expression levels/Transmembrane polypeptide (IAPP), for the specific β-cell expression. ZP3022, exendin-4, and treatment combination with exendin-4 and gastrin-17. A iasp gene expression levels which are differentially regulated in the specific β-cell/endocrine cell markers in rats treated with vehicle, ZP3022, exendin-4, and combination treatment with exendin-4 and gastrin-17. B) TTR2 gene expression levels/Transmembrane protein 37, C) Pkc1 gene expression levels/Propionyl convertase 2 (PC), D) Pkd1 gene expression levels/Propionyl convertase 3 (PC) activity.

Conclusion
- Microarray analysis revealed that ZP3022 exerted specific effects on pancreatic gene expression not observed when treating ZDF rats with either exendin-4 alone or in combination with gastrin-17. In particular, MAPK signaling pathway was observed as among the highest affected pathways.
- Moreover, rats treated with ZP3022 had a higher expression of genes encoding for the specific β-cell/endocrine cell markers, such as iasp amyloid polypeptide (IAPP), protein convertase 1/3 and -2 (PC 1/3 and -2), as well as Transmembrane protein 37 (TMEM27) compared to vehicle treated rats.
- We conclude that ZP3022 may have therapeutic potential in the prevention/delay of β-cell dysfunction.

ZEALAND PHARMA A/S
ADA, June 5-9, 2015 - Boston - MA

Figure 3. Summary of the number of differentially expressed transcripts and Venn diagram of the differentially expressed transcripts between the vehicle group and ZP3022, exendin-4 or combination with exendin-4 and gastrin-17 group. A) Differentially expressed genes between the vehicle group and each of the three compound groups (p < 0.01). B) Venn diagram of the differentially expressed genes at significance level (p < 0.01) between the three treatment groups vs. the vehicle treated animals. Numbers indicate the number of up-regulated (red) and down-regulated (black) genes after the slash, n=6-8.