Selection of the dual GCGR/GLP-1R agonist BI 456906 as a clinical candidate based on *in vitro* and *in vivo* potency as well as *in vivo* target engagement biomarkers

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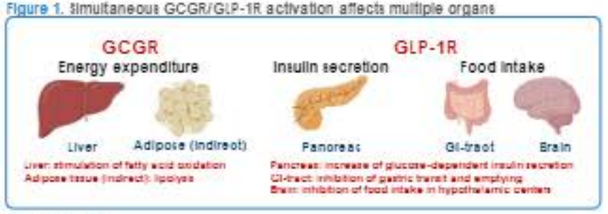
Objective

Simultaneous agonism of the receptors for GLP-1 (GLP-1R) and glucagon (GCG-R) may represent a new therapeutic approach for obesity[1], by reducing energy intake and increasing energy expenditure, respectively, with the potential for enhanced weight loss beyond those of GLP-1R agonists. This is supported by clinical investigations of oxyntomodulin, a human gut hormone with dual agonism to GLP-1R and GCG-R, which causes a significant reduction in weight by regulating both, appetite and increasing energy expenditure (EE)[2]. However, its clinical application is limited due to a short half-life. Here we describe the *in vitro* and *in vivo* profile for three GCGR/GLP-1R dual agonists BI 456906, BI 456908, and BI 456897 and their abilities to engage the GCGR and GLP-1R *in vivo* and their efficacies to lower body weight and glucose (HbA1c) in diet-induced obese (DIO) and diabetic (db/db) animals, respectively.

Figure 1. Simultaneous GCGR/GLP-1R activation affects multiple organs



Methods

- ***In vitro* pharmacology**: Receptor potencies (EC50 in nM) were determined in CHO-K1 cells of recombinant GCGR or GLP-1R expression by determining increases cAMP
- ***In vivo* pharmacology**: Experimental protocols concerning the use of laboratory animals were reviewed by a federal ethics committee and approved by governmental authorities.
- Engagement of the GCGR and GLP-1R was assessed upon single dosing of lean mice. Based on plasma FGF-21 and liver mRNA expression for nicotinamide N-methyltransferase (NNMT)[3] and improvements in oral glucose tolerance, respectively
- Subchronically, bodyweight lowering was investigated in diet-induced obese (DIO) mice together with food intake, plasma biomarkers for GCGR agonism (FGF-21; liver NNMT mRNA) and oral glucose tolerance for GLP-1R. Glucose lowering efficacy was determined in diabetic mice (male db/db at the age of 7-8 weeks; randomized for comparable baseline HbA1c) after four weeks of dosing compared to selective GLP-1R agonist.

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Disclosure: All authors are employees of Boehringer Ingelheim.

Results

Table 1. Potencies of BI 456906, BI 456908, and BI 456897 compared to the selective GLP-1R agonist Semaglutide in cells with recombinant (CHO-K1) receptor expression and improvements in oral glucose tolerance (oGTT) and plasma FGF-21 and liver NNMT mRNA upon single dosing in lean mice

Peptide	hGLP-1R	hGCGR	oGTT			Plasma FGF21		NNMT Liver mRNA	
	EC50 [nM]	EC50 [nM]	ED50 [nmol/kg] (same peptide dose)			x-fold CTL [nmol*l/g]		x-fold CTL [nmol/g]	
			2h	24h	48h	10	30	10	30
Semaglutide	0.22	>300	0.19	2.5	10	3.6	3.6	1.9	0.50
BI 456897	3.5	3.62	2.8	15	12	0.91	4.7	7.3	16
BI 456906	0.41	0.81	2.8	7.2	12	1.0	0.80	2.0	3.5
BI 456908	0.63	1.0	2.8	4.7	8.8	1.5	3.1	2.8	3.4

Figure 2. Body weight lowering efficacy (% from baseline) for BI 456906 (A), BI 456908 (B), and BI 456987 (C) after 4 weeks of treatment in diet induced obese mice. Target Engagement for the GCGR and GLP-1R was determined by plasma FGF-21 (D), liver NNMT-mRNA (E) and improvements in oral glucose tolerance (F-H)

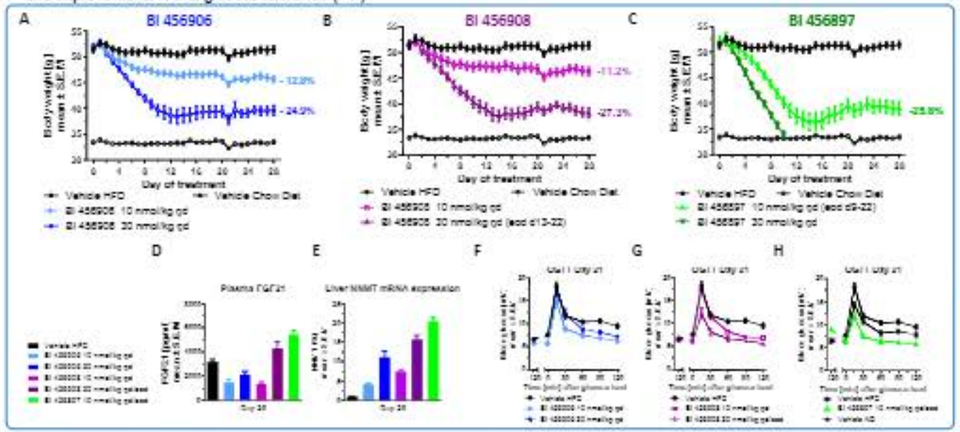


Figure 1. Target Engagement for the GCGR and GLP-1R was determined upon acute, single dosing in lean mice by plasma FGF-21 and liver NNMT-mRNA and improvements in oral glucose tolerance, respectively for 19 GCGR/GLP-1R agonists.
A) Correlation of *in vitro* GCGR potency to increase plasma FGF-21
B) Correlation of *in vitro* GCGR potency to increase liver NNMT mRNA
C) Correlation of *in vitro* GLP-1R potency to improve oral glucose tolerance compared with the selective GLP-1R agonist Semaglutide.

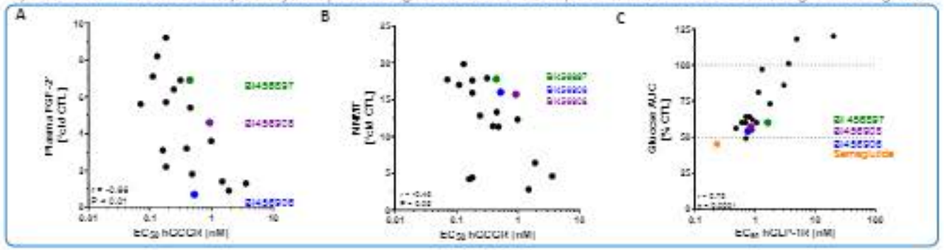
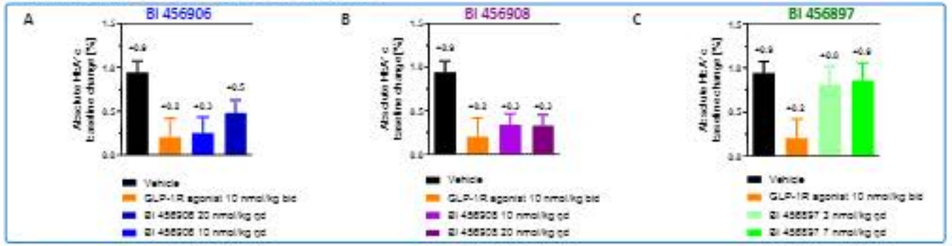


Figure 3. Glucose lowering efficacy for BI 456906 (A), BI 456908 (B), and BI 45897 (C) based on HbA1c upon four weeks of treatment in db/db mice compared to a selective GLP-1R agonist



Conclusion

- Correlation from *in vitro* to *in vivo* can be assessed for GCGR and GLP-1R by acute target engagement biomarkers such as plasma FGF-21 and liver NNMT mRNA and oral glucose tolerance tests, respectively
- The different acute profiles for BI 456906, BI 456908, and BI 456897 to engage the GCGR translate into different efficacies regarding their body weight and glucose lowering efficacies
- BI 456906 was selected as a clinical candidate based on its robust body weight lowering efficacy[3] and providing glycemic control that was comparable to a selective GLP-1R agonist

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