INVITED COMMENTARY

Glucagon receptor as a drug target: A witches' brew of eye of newt (peptides) and toe of frog (receptors)

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Glucagon has a noble history in the annals of metabolic disease,¹ even though, to a layperson, insulin is its more famous counter-regulatory partner. For decades medical students have been taught that glucagon raises blood glucose by increasing hepatic glucose output and that alleviation of hypoglycaemia is its primary function.² Thus, inhibition of glucagon secretion³ or action⁴ are logical approaches to the development of therapeutics that improve glycaemic control in both type 2 and type 1 diabetes mellitus; indeed, this strategy has been pursued for nearly 4 decades.

The situation, however, is complex. The proglucagon gene product can be cleaved at various points by specific prohormone convertases to yield several bioactive peptides, including glucagon, in a tissue-selective manner.¹ Prohormone convertase 2 is expressed in pancreatic islets to generate mainly glucagon in α cells. By contrast, prohormone convertase 1/3 processes proglucagon in the gut and brain to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), glicentin and oxyntomodulin. These boundaries are not absolute, however, and the relative abundance of these peptides in a tissue, and therefore its secretory potential, can vary depending on the differential expression of the processing enzymes.⁵ There is evidence that GLP-1 is expressed in pancreatic islets,^{6,7} while glucagon may be secreted by enteroendocrine cells in the gut.⁸ Enteroendocrine cells also express other peptides such as peptide-tyrosine-tyrosine (PYY),^{9,10} and it now appears that secretion can be modulated by feedback inhibition by homotypic (GLP-1 reducing GLP-1 secretion) as well as heterotypic mechanisms (GLP-1 reducing PYY secretion).^{11,12}

Glucagon receptor pharmacology is also multifaceted. The classic glucagon receptor¹³ is a member of the class B G-protein-coupled receptor (GPCR) family,¹⁴ and its structure has been reported recently.^{15,16} In classic experiments, glucagon was shown to elevate intracellular G-protein-coupled adenylyl cyclase activity and increase cAMP levels¹⁴; however, as with other GPCRs, the intracellular signal cascade can be modulated by other actions of G-proteins. In addition,

the glucagon receptor can be desensitized after activation by internalization, and accessory proteins, such as β -arrestin and receptor-activity-modifying proteins (RAMPs), can modulate recycling of receptors to the cell surface.¹⁷ To add complexity to this brew, not only can glucagon signal via the classic glucagon receptor,¹⁴ but it can also interact with the "GLP-1" receptor with an affinity comparable to that of GLP-1 itself.^{18,19} Indeed, oxyntomodulin, essentially a glucagon molecule with a short C-terminal extension, can also signal both via the glucagon²⁰ and GLP-1 receptors.²¹ Recently the oxyntomodulin-GLP-1 receptor interaction has been shown to exhibit biased agonism,²² and this may also apply to glucagon-GLP-1 receptor signalling.

Thus, glucagon fits into a complex "systems biology" network of ligands and targets²³ in which it can interact with 2 types of receptors with the capacity to generate a range of exquisitely subtle responses via biased signal transduction, as well as having the potential to alter the secretion of other bioactive hormones. This is the broad context within which to consider the report in the current issue of *Diabetes, Obesity and Metabolism* by Kostic et al.²⁴ on the results from an elegant first-time-in-human (FTIH) study with the glucagon receptor-blocking monoclonal antibody REGN1193. More specifically, previous studies with small molecule glucagon receptor antagonists and antisense molecules (Table 1) have uncovered good, bad and ugly aspects in humans (Table 2)²⁵; therefore, the essential question for drug development is whether these features can be separated sufficiently to open a wide therapeutic index, and allow safe long-term use. Small molecules targeting the glucagon receptor have both common structural elements and molecule-specific motifs that can confound the interpretation of adverse event profiles. Comparing the results from totally different molecular classes, small molecule-, antibody- and antisense-based approaches, therefore, allows some parsing of effects that can reasonably be attributed to mechanism-of-action vs those that may be related to molecule-specific "toxicity." Moreover, preclinical models such as glucagon

TABLE 1 Summary of glucagon receptor blocking drugs evaluated in humans

	Structure	Status	Reference
BAY27-9955	3,5 Diisopropyl-2-(1-hydroxyethyl)-6-propyl-4' fluoro-1,1-biphenyl	Discontinued	Phase I study reported ³⁰
MK-0893		Discontinued in Phase II	31–33
MK-3577		Discontinued in Phase II	34,35
LY2409021		Discontinued in Phase II	36–39, 54
PF-06291874	Non-peptide small molecule	Discontinued in Phase II	40,41
LGD-6972	Non-peptide small molecule	Phase II	42
Ionis GCGR _{RX} antisense		Phase II	43–46a
REGN1193	Human monoclonal IgG4 antibody	Phase I	This issue ²⁴

^a Data from a Phase I study with a different antisense molecule, ISIS 325568, were reported by van Dongen et al.⁴⁷

receptor knockout mice are also helpful,²⁶ as are humans with loss-of-function glucagon receptor mutations,^{27–29} especially when the biology and pharmacology are consistent and receptor mutants recapitulate the effects of glucagon receptor blockade in humans.

REGN1193 is a monoclonal antibody that blocks the glucagon receptor with a half maximal inhibitory concentration (IC₅₀) of ≈68 pM, as estimated from the inhibition of glucagon signalling in a cell-based reporter assay. This antibody binds with high affinity to

mouse, rat, monkey and human glucagon receptors, allowing translation of pharmacodynamics across species. REGN1193 has been administered to diabetic and obese mice and diabetic monkeys⁴⁸ and data from these studies were used to select doses for evaluation in the present FTIH study. In this randomized, placebo-controlled FTIH study, REGN1193 was administered by intravenous (i.v.) infusion to healthy subjects as single doses ranging from 0.05 to 0.6 mg/kg. In addition to safety and pharmacokinetic measurements, the authors

TABLE 2 Comparison of adverse effects reported in human studies with glucagon receptor-blocking drugs

Adverse events reported in human studies	REGN1193	Glucagon receptor antagonists	Glucagon receptor antisense
ALT/AST elevation	↑	↑	↑
LDL cholesterol	No change	↑	No increase
Hypoglycaemia (≤70 mg/dL)	↑	↑	No change
Blood pressure	No increase	↑	No increase
Body weight	No change	↑	No change
Hepatic steatosis	?	↑	?
Circulating gluconeogenic amino acids ^a	↑	↑	?

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low density lipoprotein.

^a In rodent studies, REGN1193,⁴⁸ glucagon receptor antagonists^{49,50} and glucagon receptor knockout²⁶ increase circulating amino acid levels, resulting in pancreatic islet α-cell hyperplasia.^{51,52}

TABLE 3 Summary of the pharmacodynamic effects of REGN1193 compared with those observed with other methods of modulating the glucagon and GLP-1 systems

	REGN1193	Glucagon receptor antagonists	Glucagon receptor antisense	Glucagon receptor knockout	Glucagon receptor agonist	GLP-1 receptor agonist
Glycaemic response to glucagon challenge	↓	↓	↓	↓	NA	?
HbA1c	No data	↓↓	↓↓	↓	?	↓
Circulating glucagon concentration	↑	↑↑	↑	↑↑	?	↓
Circulating insulin concentration	?	No change	?	No change	↑ (acute)	↑
Circulating C-peptide concentration	↓ post glucagon challenge	↑ (fasting)	?	?	↑ (acute)	↑
Circulating total GLP-1 concentration	↑ ^a	↑ ^a	↑	↑↑	↓ ⁵³	↓
Circulating GLP-2 concentration	↑	↑	?	↑	?	?
Glucose-dependent insulinotropic peptide (GIP)	No change	?	?	?	↓ ⁵³	?
Circulating PYY concentration	?	?	?	?	?	↓
Circulating bile acid metabolites	?	↑	?	?	?	?

Abbreviation: NA, not applicable.

^a No consistent change in active GLP-1.

tracked pharmacodynamics directly using the inhibition of the hyperglycaemic response to a standardized glucagon challenge (0.8 mg i.v.). Onset-of-action was investigated using a pharmacologically active 0.3 mg/kg dose and the standardized glucagon stimulation test. Development of anti-drug antibodies was monitored over the 15-week observation period.

REGN1193 exhibited markedly non-linear clearance, resulting in half-life estimates of 0.5 days for the 0.05 mg/kg dose to 4 days for the 0.6 mg/kg dose. Anti-drug antibody formation was unremarkable and did not contribute to the variable pharmacokinetics. The pharmacodynamic profile observed is summarized in Table 3. As expected from the non-clinical data, the higher doses of REGN1193 blocked the elevation of plasma glucose and C-peptide seen with the glucagon challenge, effects consistent with the reported actions of the non-peptide small molecule antagonists and antisense molecules listed in Table 1. The blocking effect of REGN1193 was evident as early as 6 h post-dose and persisted for at least 3 weeks. Previous work with the small molecule MK-0893 demonstrated predictable glucose-lowering in subjects with diabetes based on the degree of inhibition of glucagon-mediated hyperglycaemia³¹; however, it is essential that the effects of REGN1193 be tested in subjects with type 2 diabetes to estimate clinical effects and allow comparison with the ≥1% glycated haemoglobin (HbA1c) reductions observed with the small molecule antagonists^{31,32,34–36,40,54} and antisense molecules.⁴³

While the “good” appears to be very good in terms of HbA1c reduction, inhibition of glucagon signalling in humans appears to carry liabilities (Table 2), and this has led to the termination of several drug development programmes in phase II or before (Table 1). It appears that all interventions in rodents that have interrupted glucagon

receptor signalling have resulted in pancreatic islet α -cell (and δ -cell) hyperplasia to a greater or lesser extent^{26,49,50,55,56} related to the degree of interruption of a liver signal.⁵⁷ Recently, a causal link has been established in glucagon receptor knockout mice between increased circulating amino acid levels, in particular glutamine, and the α -cell hyperplasia.^{51,52} In this context, it is interesting to note that administration of 75 mg/kg REGN1193 for 8 weeks to normoglycaemic cynomolgus monkeys did not increase pancreatic α -cell area, but did increase circulating amino acids, including glutamine; for comparison, 20 mg/kg was pharmacologically active in diabetic monkeys.⁴⁸ This may be evidence of a difference in responsivity between murine and monkey islets or insufficient “dose $\times$ time” or a combination of both, but it is notable that humans with loss-of-function glucagon receptor mutations demonstrate α -cell hypertrophy.^{27–29} The data relating to reversible increases in ALT and AST (without elevation of bilirubin) appear to be consistent across all glucagon receptor blocking modalities and there is now a report that LY240921 increases liver fat measured by MRI.³⁷ As regards the elevation of LDL cholesterol initially observed with MK-0893 and MK-3577,^{32,34} this appears to correlate with the magnitude of glucose-lowering in people with type 2 diabetes, suggesting a mechanism-based association, perhaps through altered absorption of cholesterol.⁵⁸ Initially, variable data were presented for LY240921 (no significant increase in LDL cholesterol after 12 or 24 weeks of treatment,³⁶ elevation of total cholesterol⁵⁴), but a more recent full publication clearly shows that 6 months of treatment with the compound increases circulating total cholesterol accompanied by a non-significant increase in LDL cholesterol compared with placebo.³⁷ This is consistent with results using a different molecule, PF-06291874, in which the highest dose

(150 mg) administered for 4 weeks significantly increased LDL cholesterol.⁴⁰ No significant lipid effects have been described with the small molecule LGD-6972⁴² or the antibody REGN1193, but it is not yet clear whether these are true differences from the other molecules or simply a reflection of shorter duration of dosing or the populations studied. LY240291 and MK-0893 increased body weight and blood pressure,^{31,32,37} and there were trends observed with PF-06291874,⁴⁰ but again these have not been observed with LGD-6972 or REGN1193. Interestingly, preliminary communications indicate that up to 14 weeks of dosing with the antisense lonis GCGRx was not associated with increases in LDL cholesterol, body weight or blood pressure,⁴⁴ raising mechanistic questions about these phenomena.

Although the development of glucagon receptor antagonists is proving a thorny issue, it is yielding insights into the witches' brew of the glucagon–glucagon receptor system and related peptides. Kostic et al.²⁴ describe actions of REGN1193 on various peptide biomarkers and these are compared across classes of drugs in Table 3. For example, the universal observation of elevated glucagon secretion when the receptor is blocked raises the possibility that there may be significant signalling through the GLP-1 receptor,¹⁸ as suggested by receptor knockout studies.¹⁹ Overlying this is the interesting possibility that there is differential feedforward stimulation (insulin/C-peptide) and feedback inhibition (oxyntomodulin, glicentin, PYY, and even GLP-1 and GLP-2) of the secretion of bioactive peptides that may vary greatly depending on how much the receptor signalling is disturbed and whether this is being done in a “biased” manner. Some caution is prudent, therefore, when attributing the pharmacological and safety profiles of glucagon receptor blockers solely to their action at the hepatic glucagon receptor, and constant comparison across molecules is justified. As an example, circulating GLP-2, a peptide that stimulates growth of the gastrointestinal tract,^{59,60} is elevated when the glucagon receptor is blocked, indicating that long-term safety will require monitoring not only for the potential effects of absence of glucagon action, but also for actions attributable to accompanying changes in related bioactive peptides.

In conclusion, the road to a useful medicine based on blocking glucagon signalling appears steep and sticky, but the subtlety of the system may provide some hope that a golden key can be found if investment in drug discovery is maintained.

Conflict of interest

D. D'A. has served as a consultant for Eli-Lilly and Co., Novo Nordisk Inc. and Janssen Pharmaceuticals Inc, has received research grant support from Merck and Co., and is an investigator in a clinical trial sponsored by Ligand Pharmaceuticals, Inc. D. J. N. has no conflict of interest to declare.

Author contributions

D.J.N. wrote the first draft. D.J.N. and D.D'A. reviewed the drafts and final submitted version.

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