

GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP): DEVELOPMENT OF DPIV-RESISTANT ANALOGUES WITH THERAPEUTIC POTENTIAL

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1. INTRODUCTION

Glucose-dependent Insulinotropic Polypeptide (GIP₁₋₄₂; Figure 1) is a gastrointestinal hormone that is released in response to nutrient intake and stimulates insulin secretion in a glucose-dependent manner^{1,2} and was the first established hormonal component (incretin) of the enteroinsular axis identified. Subsequently, intestinal products of the proglucagon gene, GLP-1₇₋₃₆ and GIP-1₇₋₃₇ (GLP-1), were also shown to share the ability of stimulating insulin secretion and the dual actions of GIP and GLP-1 are widely considered to be the major hormonal contributors to intestinal regulation of pancreatic endocrine function.

There are two major defects in type 2 diabetes that contribute to the hyperglycemia that is characteristic of the disorder: defective responsiveness to glucose and insulin resistance. Current therapies for type 2 diabetics generally involve dietary control plus stimulants of insulin secretion (e.g. sulfonylureas) and/or insulin sensitizers (e.g. metformin, thiazolidinediones). However considerable numbers of patients become resistant to sulfonylurea action and many eventually become insulin-dependent. As a consequence, there is increasing interest in developing alternative methods for stimulating endogenous insulin secretion. Among these, there has been a major emphasis

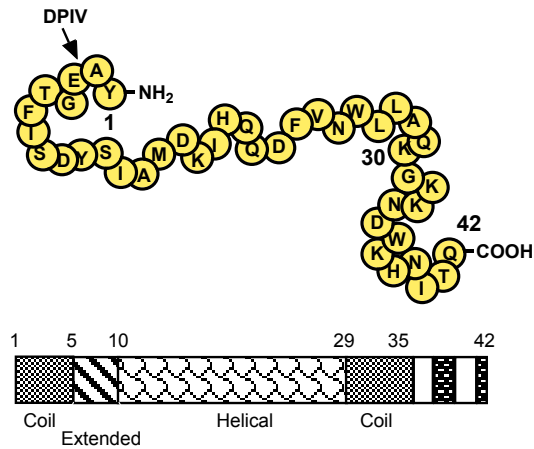


Figure 1. Predicted Secondary Structure of GIP

Secondary structure was predicted by the Gascuel and Golmard Basic Statistical Method using PCGENE. The site of DPIV-cleavage is shown.

on long-acting analogues of GLP-1³ and, more recently, DPIV inhibitors with little interest shown in GIP⁴. The current short review focuses on the underlying reasons for this disinterest and attempts to provide a balanced view on the potential for DPIV-resistant analogues of GIP in the treatment of diabetes.

2. WHY DEVELOP GIP ANALOGUES?

GIP potently stimulates insulin secretion in humans in a glucose-dependent manner². Additionally, recent studies have demonstrated that it stimulates beta cell mitogenesis and inhibits apoptosis^{5,6}. GIP₁₋₄₂ and GLP-1 are equally insulinotropic⁷ and conclusions of a much greater potency of GLP-1 can be attributed to comparative studies with batches of synthetic GIP with low biological activity^{7,8}. In addition to the common physiological actions exhibited by GIP and GLP-1, GIP also exhibits discrete actions. For example, GIP demonstrates both lipolytic and lipogenic activity on fat cells^{4,9}. Although the significance is not clear, one possibility is that GIP stimulates lipolysis during fasting conditions, thus providing tissues, including the β -cell, with fatty acids that are essential for their normal function⁹. During a meal, incretins stimulate insulin secretion and GIP may then act in a lipogenic manner, as insulin inhibits the lipolytic pathway.

3. PROBLEMS WITH GIP ANALOGUE DEVELOPMENT

The two major arguments brought against developing analogues of GIP for

therapeutic use are that it is rapidly degraded in the bloodstream and that type 2 diabetic patients exhibit resistance to GIP action. The first indication that GIP was a target for DPIV degradation was obtained in HPLC studies on partially purified fractions from porcine intestinal extracts¹⁰. A peptide corresponding to GIP₃₋₄₂ was identified and it was suggested that enzymatic cleavage resulted in formation of the N-terminally truncated molecule¹¹. GIP₃₋₄₂ was shown to be non-insulinotropic in the perfused rat pancreas¹⁰ and isolated islets. Mentlein et al¹² first reported that GIP₁₋₄₂ was a substrate for DPIV, with release of Tyr¹-Ala². Serum-degradation of GIP by DPIV was established using the inhibitor, Lys-Pyrrolidide¹². The *in vivo* importance of DPIV-mediated degradation of GIP was demonstrated independently by following ¹²⁵I-GIP₁₋₄₂ conversion to ¹²⁵I-GIP₃₋₄₂ following i.v. injection into anaesthetized rats¹³.

Identification of the second problem, GIP resistance, has its origins in studies by Perley and Kipnis¹⁴ in which they observed a greatly reduced incretin effect in type 2 diabetes. Studies were later performed to determine whether pathophysiological changes in GIP secretion contributed to this defect^{1,2} and whether responsiveness to incretins was compromised. Several groups have now shown blunted responses to GIP infusion in type 2 diabetes, although the degree of resistance observed differs between groups¹⁵⁻¹⁷. It is important to note that all of the studies to date have employed infusion conditions designed to produce circulating levels in the physiological range.

4. DEVELOPMENT OF DPIV-RESISTANT GIP ANALOGUES

The protocol that we have used for screening for DPIV-resistant analogues over the past few years can be summarized^{18,19}:

1. Solid phase peptide synthesis and screening for DPIV-resistance using Maldi-TOF mass spectrometry,
2. Competitive binding assays using CHO-K1 cells transfected with the GIP receptor (GIP-R1 cells) to establish binding constants (IC₅₀ values).
3. Measurement of analogue-induced cyclic AMP production in CHO-K1 cells and determination of maximal response and EC₅₀ values.
Analogues with promising characteristics in 1-3:
4. Determination of analogue-induced insulin secretion from insulinoma cells (□TC-3, INS-1) and/or isolated perfused rat pancreas.
5. In vivo bioassay during an oral (OGTT) or intraperitoneal (IPGTT) glucose tolerance test in normal and diabetic rats.

Design of the GIP analogues was based on previous studies with other

members of the secretin-glucagon family of peptides and on the basis of knowledge regarding the substrate specificity requirements of DPIV. The N-terminus of GIP is extremely sensitive to change and removal of the first two amino-terminal residues (GIP₃₋₄₂) results in a peptide that exhibits reduced receptor affinity in competitive binding studies and is completely devoid of the ability to stimulate cAMP production or affect glucose excursions and insulin profiles *in vivo*. Therefore, to generate DPIV-resistant GIP analogues, peptides were generated with modifications or substitutions of amino acids in positions 2 and 3. Additionally, for the majority of syntheses, the GIP₁₋₃₀ backbone was used, rather than the intact peptide. Using the above screening procedure the following GIP analogues were identified as being completely or moderately DPIV-resistant and worthy of further study¹⁸:

COMPLETELY RESISTANT	MODERATELY RESISTANT*
[D-Ala ²]GIP ₁₋₄₂ and [D-Ala ²]GIP ₁₋₃₀ [Pro ²]GIP ₁₋₃₀	Val ²]GIP ₁₋₃₀
[Tyr ¹ -Ala ² -(CH ₂ NH)Glu ³]GIP ₁₋₃₀ [#]	[Gly ²]GIP ₁₋₃₀
[(P)Ser ²]GIP _{1-30NH2}	[Ser ²]GIP ₁₋₃₀
[Pro ³]GIP _{1-30NH2}	[D-Tyr ¹]GIP ₁₋₃₀
[N-MeGlu ³]GIP ₁₋₃₀	[PPa ¹]GIP ₁₋₃₀ ^{##}
	[D-Glu ³]GIP ₁₋₃₀

*t_{1/2} = 137-298 min with purified enzyme [#] [Tyr¹-Ala²-(CH₂NH)Glu³]GIP_{1-30NH2}: reduced peptide bond between Ala² and Glu³ ^{##}PPa = ((Desamino Tyr¹(phenylpropionic acid)

Competitive binding and cyclic AMP stimulation studies were performed on GIP-R1 cells with all peptides. In general, when examining binding affinity, the amino-terminus of GIP was fairly tolerant of amino acid substitution or modification. With [PPa¹]-, [D-Tyr¹]-, [D-Ala²]- and [D-Glu³]-GIP little or no change in binding affinity was observed. Substitutions of [T¹A²-(CH₂NH)], [Gly²], and [Ser²] were well tolerated with respect to binding affinity, and binding of [Val²]-, [Pro³]- and [N-MeGlu³]-GIP was only modestly reduced. However all peptides exhibiting complete DPIV resistance, apart from [D-Ala²]GIP, displayed dramatically reduced cyclic AMP stimulating ability. [T¹A²-(CH₂NH)]-, [Pro³]- and [N-MeGlu³]-GIP were not even capable of achieving maximal levels. [(P)Ser²]GIP showed both greatly reduced binding affinity and cAMP production.

From these binding and cyclic AMP data, [D-Ala²]GIP was concluded to have the greatest potential for further peptide development²⁰. Both [D-Ala²]GIP₁₋₃₀ and [D-Ala²]GIP₁₋₄₂ were completely resistant to DPIV degradation for over 24 hours, and had minimal changes in receptor binding. When tested *in vivo*, [D-Ala²]GIP reduced glycaemic excursions in Wistar rats and both lean and obese Vancouver diabetic Zucker (VDZ) rats to a greater extent than native GIP.

This was associated with enhanced early phase insulin release in lean animals, and in diabetic rats where first phase insulin release is compromised, augmentation over the entire insulin time-course was observed. This is of particular interest since, in contrast to the lack of insulin response in fatty VDZ rats when GIP was administered to approximate physiological levels²¹, insulin responses could be induced with pharmacological doses.

Although [Ser²]GIP_{1-30NH₂} was only moderately resistant to DPIV degradation (~137-686 min), it had favourable binding and signaling characteristics at the cloned GIP receptor. In studies on the degradation of glucagon²², which normally has a serine in position 2, serine phosphorylation resulted in complete resistance to purified DPIV, and this molecule was rapidly dephosphorylated in serum. We therefore examined the effect of phosphorylation of Ser² on the characteristics of GIP_{1-30NH₂}. (P)Ser²] GIP_{1-30NH₂} was completely resistant to DPIV. Although the affinity of [(P)Ser²]GIP was reduced 21-fold and the concentration-response curve for cAMP production was right shifted (433-fold), when [Ser²]GIP_{1-30NH₂} was administered sc during a OGTT, it resulted in a slightly more pronounced reduction in the glycaemic profile than GIP_{1-30NH₂}, and an enhanced insulin time-course. Higher doses of [(P)Ser²] GIP resulted in greater reduction in the glycemic profile than GIP_{1-30NH₂}, and significantly enhanced insulin responses. Both [D-Ala²]GIP and [(P)Ser²]GIP are therefore promising analogues and, along with [Tyr¹-Glucitol]GIP²³, may well prove useful for improving glucose tolerance in humans.

5. DEVELOPMENT OF TRUNCATED GIP ANALOGUES

GIP is the largest polypeptide of the glucagon-secretin family, with 42 amino acids, as compared to 30 in GLP-1₇₋₃₆. It would clearly be advantageous to develop truncated forms of the molecule that retain biological activity, and we performed structure-activity studies with the view to designing such analogues.

Evidence has been presented for the existence of multiple domains in GIP₁₋₄₂¹⁹. Early studies showed that it is possible to truncate the C-terminus with retention of biological activity; synthetic GIP₁₋₃₈ and GIP₁₋₄₂ were shown to be equally insulinotropic^{1,2}. Equal potency to GIP₁₋₄₂ has been demonstrated for GIP₁₋₃₁ and GIP₁₋₃₀ in stimulating cyclic AMP production in insulinoma cells⁸ and insulin release from the perfused pancreas. However GIP_{1-30NH₂} lacks gastric effects, although it is unclear whether this is due to the existence of a second GIP receptor, an alternatively spliced receptor, or differential ligand recognition and coupling of the receptor in gastric cells.

The insulinotropic domain of GIP has been localized to residues 19 to 30, consistent with partial retention of insulinotropic activity of GIP₁₉₋₃₀, GIP₁₅₋₄₂ and GIP₁₇₋₄₂^{1,19}. Residues 27-30 may be important for biological activity, as GIP₁₋₂₇ and GIP₁₋₂₈ are devoid of insulinotropic potency. Computer assisted

secondary structure analysis of GIP predicts an alpha helical region between residues 10 and 29 (Fig. 1). Hence, it is possible that this helical structure is important for biological activity. Antagonism of the GIP receptor has been demonstrated with N-terminally truncated peptides, GIP_{6-30NH₂}, GIP_{7-30NH₂}, and GIP_{10-30NH₂}, and the complete high affinity binding domain of GIP resides between residues 6 and 30²⁴. Recently, we obtained evidence for a bioactive domain of GIP residing in residues 1-14²⁰. In binding studies, bioactivity of N-terminal GIP fragments was restricted to GIP₁₋₁₄ and amidated forms of GIP₁₋₁₃ and GIP₁₋₁₅. GIP_{1-14NH₂} exhibits low, but significant, insulinotropic activity. Using a bioassay, both GIP_{1-14NH₂} and, to a lesser extent, GIP_{19-30NH₂} reduced excursions in glycaemia in an equivalent manner to GIP_{1-42OH}, although 100-fold greater doses were needed²⁰.

In recent studies, an alanine scan of GIP_{1-14OH} was performed to identify key residues contributing to biological activity. Substitution of any residue of the 1-14 primary sequence resulted in significantly reduced binding affinity, with the notable exception of [Tyr¹³]GIP_{1-14OH}. The substituted analogs Ala¹, Ala³, Ala⁴ or Ala⁵ were devoid of biological activity indicating that these residues are particularly important for conferring structure optimal for binding. [D-Ala²] substitution of GIP_{1-14OH} was also not well tolerated.

Given that GIP₁₋₁₄ and GIP₁₉₋₃₀ both demonstrate receptor binding ability, and that the high affinity binding domain of GIP resides within residues 6 to 30²⁴, it is likely that multiple contact residues contribute to high affinity receptor binding. Additionally, the two N-terminal residues may either interact with or be in close proximity to the core region (possibly indicating the presence of a functional hinge in the alpha helices), resulting in receptor activation. Using this information it may be possible to develop more potent analogues.

CONCLUSION

Although type 2 diabetic patients exhibit resistance to GIP when the peptide is administered in doses that result in circulating levels approximating those found physiologically, it is likely that DPIV-resistant forms of the peptide administered in pharmacological doses will prove to be effective in improving glucose tolerance. Additionally, in view of recent studies showing that GIP receptor knockout mice are resistant to diet induced obesity²⁵, it is possible that GIP antagonists will prove useful in obesity treatment.

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