Peptide-based GLP-1/glucagon co-agonists: A double-edged sword to combat diabesity

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A R T I C L E   I N F O

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A B S T R A C T

Diabesity is a new term for obesity-dependent diabetes, which is also associated with cardiovascular and other comorbidities with rising epidemic. Traditional treatments (sulfonylureas and thiazolidinediones) of diabetes are associated with weight gain, except metformin. Newer agents such as glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and Sodium glucose co-transporter 2 inhibitors (SGLT2i) are causing a modest weight reduction, whereas dipeptidyl peptidase-4 inhibitors (DPP-4i) are weight neutral. Oxyntomodulin, a native GLP-1/glucagon receptor agonist produced a superior weight loss and anti-hyperglycemic effects in obese mice and humans. Recent findings with synthetic dual GLP-1/glucagon receptor agonists have shown a good weight loss and anti-hyperglycemic profile in diet-induced obese (DIO) mice. Targeting combinations of GLP-1 receptor and glucagon receptor simultaneously with a single peptide may be the better strategy to achieve marked weight loss and considerable glycemic control in diabesity. Cardiovascular safety is very important with new antidiabetic agents due to rosiglitazone controversy. Current on-going clinical trials will clarify the cardiovascular effects of incretin-based therapies in near future. Based on current knowledge and rapid progress in the field, there is a strong possibility that the GLP-1/glucagon receptor co-agonists will likely be the new therapeutic treatment for diabesity for decades to come.

Introduction

Obesity is the most critical factor for the development of type 2 diabetes mellitus (T2D). According to the World Health Organization (WHO), diabetes and obesity are most challenging twenty-first century epidemic [1]. There is a strong pathophysiological link between diabetes and obesity and therefore the term “diabesity” was coined [2]. It is estimated that the majority (~80–90%) of all individuals with T2D are obese, which indicate the role of insulin resistance, pancreatic dysfunction, hepatic glucose production and adiposity as risk factors for diabetes [3]. Modulation of gut hormones is one of the strategy to control hyperglycemia in T2D. Gut hormones also play an important role in appetite and food intake [4]. The major drawback of the conventional treatments (sulfonylureas, thiazolidinediones) for T2D is weight gain except metformin [5]. Bariatric surgery showed dramatic improvement in diabesity and associated comorbidities. However, surgery leads to complex alterations in the level of physiologically important gut hormones [6]. Currently available drugs for hyperglycemia are either caused moderate weight loss or body weight neutral. Therefore, there is an urgent need to develop a drug which shows dramatic body weight loss and marked glucose control in patients suffering from diabesity.

Glucagon, old neglected peptide hormone, but important in energy expenditure

Glucagon is a 29 amino acid peptide hormone that is isolated and purified even before insulin. Glucagon is biologically potent hormone involved in glycemic control and studied extensively in rodents, non-rodents and humans for various biological activities. Proglucagon, a 160 amino acid polypeptide produces many biologically active peptides including glucagon-like sequences at its C-terminal, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) [7]. Human proglucagon 33–61, is synthesized and released from pancreatic alpha cells and acts on glucagon receptor. The human glucagon receptor is a highly conserved G-protein-coupled receptor (GPCR) with 485 amino acid protein with a 143-amino-acid extracellular N-terminal. The N-terminal sequence of the human glucagon receptor has 97% homology with...
rats. Glucagon receptors are highly expressed in liver, but also expressed in pancreas, kidney, heart, adipose tissue and gastrointestinal tract (GIT). The activation of glucagon receptor leads to activation of adenyly cyclase, which increases intracellular Cyclic adenosine monophosphate (cAMP) followed by increase in inositol 1,4,5-trisphosphate (InsP3) and calcium. Glucagon has many physiological effects such as stimulation of hepatic glucose production by glycogenolysis, regulation of hepatic ketogenesis, regulation of bile acid metabolism and the satiating effect via vagal nerve. Therapeutically, glucagon has been used for severe acute hypoglycemia where oral or intravenous glucose is not effective. Glucagon receptor activation reduces food intake and promotes lipolysis and weight loss in animals and humans. Receptors for glucagon and GLP-1 are structurally related but both hormones showed the opposite effect in controlling glucose [8].

### Table 1

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Duration of action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1 (7–36)</td>
<td>t½ ~2 min (ultra-short acting)</td>
<td>Native GLP-1 rapidly degraded primarily by DPP-4</td>
</tr>
<tr>
<td>Exenatide/exendin-4 (Byetta®; originally developed by Amylin now distributed by AstraZeneca)</td>
<td>t½ ~2.4 h</td>
<td>53% homology with native GLP-1</td>
</tr>
<tr>
<td>Liraglutide (Victoza®; Novo Nordisk)</td>
<td>t½ ~11–13 h</td>
<td>Short-acting, BID</td>
</tr>
<tr>
<td>Semaglutide (Novo Nordisk)</td>
<td>t½ ~160 h</td>
<td>Once-weekly s.c. injection</td>
</tr>
<tr>
<td>Tasipoglutide (Roche/Ipsen)</td>
<td>Long-acting</td>
<td>Once-weekly injection</td>
</tr>
<tr>
<td>Lixisenatide (Lyxumia®; Sanofi/Zealand)</td>
<td>t½ ~3 h</td>
<td>Once-weekly injection</td>
</tr>
<tr>
<td>Exenatide-LAR (Bydureon®; developed by Amylin/Lilly/Alkermes—now product of AstraZeneca)</td>
<td>t½ ~5–6 days</td>
<td>Once-weekly formulation of exenatide</td>
</tr>
<tr>
<td>Albiglutide (Tanzeum®; GlaxoSmithKline)</td>
<td>t½ ~6–8 days</td>
<td>Fusion product of GLP-1 dimer with albumin</td>
</tr>
<tr>
<td>Dulaglutide (Trulicity®; Eli Lilly)</td>
<td>t½ ~4 days</td>
<td>Fusion protein with two GLP-1 peptides</td>
</tr>
<tr>
<td>Langenatide (Hannmi Pharmaceuticals)</td>
<td>t½ ~150 h</td>
<td>Fused Exenatide analog with PEG</td>
</tr>
<tr>
<td>Exenatide minipump/ ITCA 650 (Intarcia)</td>
<td>Once in 6 months /once yearly s.c. implant</td>
<td>Exenatide in matchstick size osmotic pump</td>
</tr>
<tr>
<td>Glymera (PB1023; PhaseBio Pharmaceuticals Ltd) VRS 859 (Exenatide-XTEN; Diartis Pharmaceuticals)</td>
<td>Once weekly injection</td>
<td>GLP-1 analog fused to elastin-like peptide</td>
</tr>
<tr>
<td>TTP273 (TransTech Pharma is now known as vTv Therapeutics)</td>
<td>Orally bioavailable</td>
<td>Long acting PEGylated exenatide</td>
</tr>
<tr>
<td>ZYQG1 (ZYDUS Cadila)</td>
<td>Orally bioavailable</td>
<td>New small molecule GLP-1 RAs using TTP Translational® Technology</td>
</tr>
<tr>
<td>ARI-1732TS (Arisaph Pharmaceuticals)</td>
<td>t½ ~12 h in dogs</td>
<td>In Phase I clinical trial</td>
</tr>
</tbody>
</table>

Abbreviations: s.c.=subcutaneous, BID = twice daily.

From [10,11].

Recently, Intarcia has announced successful cardiovascular safety results in Phase 3 FREEDOM-CVO trial for ITCA 650—exenatide osmotic minipump for the treatment of T2D (Source: www.intarcia.com/media, accessed on 12th August, 2016).

### GLP-1 agonists for diabetes with modest weight reduction activity

Intestinal mucosa secretes various peptide hormones such as gastrin, secretin, peptide YY, cholecystokinin, motilin, gastric inhibitory peptide (GIP), GLP-1 and GLP-2 [9]. GLP-1 is a 29-amino acid polypeptide incretin hormone secreted from intestinal L-cells and is a transcription product of proglucagon. GLP-1 is released into the circulation after meal intake and exerts biological activity by activation of GLP-1 receptor, a class B GPCR. GLP-1 has many biological actions, including glucose-dependent insulin secretion, glucagon suppression, delay in gastric emptying and appetite suppression [6]. Natural GLP-1 has limited therapeutic potential due to its rapid degradation by dipeptidyl peptidase-4 (DPP-4), neutral endopeptidase (NEP), plasma kallikrein or plasmin. Native GLP-1 has ultra-short half-life of ~2 min after intravenous (i.v.)
injection. Therefore, many efforts have been made to improve the efficacy and duration of action by utilizing chemical modification and/or formulation approach for the treatment of diabesity [10,11]. There are also several orally bioavailable GLP-1 receptor agonists (GLP-1 RAs) under development (Table 1).

Exenatide, a synthetic version of exendin-4, is the first GLP-1 RA approved in 2005 for the treatment of T2D. Clinically, exenatide showed 1.8–2.6 kg body weight reduction after 30 weeks and 4.4 kg after 82 weeks of BID (twice daily) subcutaneous (s.c.) administration. Exenatide and Exenatide LAR-Bydureon accompanied by similar body weight reduction data. Lixisenatide and taspoglutide were also similar to exenatide in terms of body weight reduction. Albiglutide showed moderate body weight loss probably due to large size of the molecule to penetrate blood brain barrier for CNS action [10]. Liraglutide produced 5.6 kg weight loss in obese nondiabetics and obese diabetic patients. Semaglutide showed body weight reductions up to 4.8 kg [12,13]. ITCA 650 (Intarcia) is a matchstick size osmotic mini-pump for continuous s.c. exenatide delivery. It can effectively control the blood glucose level up to one year. ITCA 650 produced body weight reductions between 2.4 and 4.2 kg in various clinical trials. [14]. Potency is very important factor to deliver a drug using this type of minipump technology because there is a need to incorporate sufficient quantity of drug into the minipump device for continuous long-term delivery. The extended release osmotic minipump technique will also be useful for extremely potent peptides such as recombinant human erythropoietin (rHuEPO) to treat renal anemia patients or any other potent cardiovascular agents to minimize early morning heart attack in heart patients. Novel GLP-1 RAs showed improvement in duration of action and glycemic control. However, there is still a need for a better molecule to produce a significant body weight loss and to control hyperglycemia for obese patients with T2D (see Table 2).

GLP-1/glucagon coagonists for diabesity, a novel workable hypothesis

Glucagon, GLP-1, and oxyntomodulin (OXM) are transcription products of proglucagon gene. The source of all three peptide hormones are similar but have different or opposite physiological role (Fig. 1). The processing of proglucagon gene is tissue dependent. Glucagon generation takes place in pancreas whereas glicentin, oxyntomodulin, GLP-1, and GLP-2 generates in intestinal L-cells. [15]. It has been reported that glucagon has beneficial effects on body fat mass, nutrient intake and energy expenditure in rodents and humans [16,17]. Oxyntomodulin (OXM), a peptide hormone secreted along with GLP-1 by intestinal L-cells after meal ingestion, is a dual agonist of the GLP-1 receptor and the glucagon receptor. It

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**Table 2**

Peptides with GLP-1/glucagon co-agonist property.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Duration of action</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyntomodulin</td>
<td>t½ ~ 12 min</td>
<td>Therapeutically not useful due to short half life</td>
</tr>
<tr>
<td>LY2944876/TT-401 (Eli Lilly)</td>
<td>Long acting</td>
<td>Under Phase II trial</td>
</tr>
<tr>
<td>ZP2929 (Zealand in collaboration with Boehringer Ingelheim)</td>
<td>Once daily s.c. injection</td>
<td>Under Phase I trial</td>
</tr>
<tr>
<td>HM2525A (Hamnu Pharmaceuticals)</td>
<td>Long acting</td>
<td>Under Phase I trial</td>
</tr>
<tr>
<td>MED0382 (MedImmune)</td>
<td>Weekly s.c. injection</td>
<td>Under Phase I trial</td>
</tr>
<tr>
<td>SAR 425899 (Sanofi)</td>
<td>Weekly s.c. injection</td>
<td>Under Phase I trial</td>
</tr>
<tr>
<td>VPD-107 (Spitfire Pharma)</td>
<td>Long acting</td>
<td>Under preclinical investigation</td>
</tr>
<tr>
<td>MOD-6030/1 (Prolor/OPKO biologics)</td>
<td>Weekly s.c. injection</td>
<td>Under preclinical investigation</td>
</tr>
<tr>
<td>IJB447 (MB-2 LLC)</td>
<td>Daily s.c. injection</td>
<td>Under preclinical investigation</td>
</tr>
</tbody>
</table>

From [21].

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**Fig. 1.** Three glucagon gene transcription product with diverse biological activity.
Critical success factors (CSFs) to develop balanced GLP-1/glucagon coagonist peptide for the treatment of diabesity.

1. Peptide should cause a substantial body weight reduction
2. Peptide should cause glucose-dependent glycemic control
3. Peptide should have prolonged 1/2 to minimize the frequency of administration
4. Peptide should have low nanomolar or picomolar potency
5. Peptide should not cause hypo or hyperglycemia at therapeutic concentrations
6. Peptide should cause minimal antibody formation and that should not affect the safety and efficacy
7. Peptide should not cause allergic reaction
8. Peptide should preferably be orally bioavailable
9. Peptide should be chemically and metabolically stable
10. Peptide should have a better safety profile, especially in terms of cardiovascualr safety
11. Peptide should be cheap to produce in bulk
12. Peptide should not be thermolabile to reduce the cost of cold chain transportation

Table 3

<table>
<thead>
<tr>
<th>Peptide should...</th>
<th>Reason</th>
</tr>
</thead>
<tbody>
<tr>
<td>cause a substantial body weight reduction</td>
<td>For effective weight management</td>
</tr>
<tr>
<td>cause glucose-dependent glycemic control</td>
<td>To control blood sugar levels</td>
</tr>
<tr>
<td>have prolonged 1/2 to minimize the frequency of administration</td>
<td>For reduced dosage and improved patient compliance</td>
</tr>
<tr>
<td>have low nanomolar or picomolar potency</td>
<td>To minimize side effects</td>
</tr>
<tr>
<td>not cause hypo or hyperglycemia at therapeutic concentrations</td>
<td>To maintain safe blood sugar levels</td>
</tr>
<tr>
<td>cause minimal antibody formation</td>
<td>To ensure long-term therapy efficacy</td>
</tr>
<tr>
<td>preferably be orally bioavailable</td>
<td>For improved patient compliance and convenience</td>
</tr>
<tr>
<td>be chemically and metabolically stable</td>
<td>To ensure safety and efficacy</td>
</tr>
<tr>
<td>have a better safety profile, especially in terms of cardiovascualr safety</td>
<td>For improved patient safety</td>
</tr>
<tr>
<td>be cheap to produce in bulk</td>
<td>To reduce production costs</td>
</tr>
<tr>
<td>not be thermolabile to reduce the cost of cold chain transportation</td>
<td>To reduce storage and transportation costs</td>
</tr>
</tbody>
</table>

is a 37 amino acid polypeptide secreted together with GLP-1 by intestinal L-cells after meal intake. Similar to glucagon OXM produces significant weight loss in humans and rodents. Weight loss activity of OXM has been observed with selective GLP-1 agonist in obese mice at equimolar doses. It has been found that OXM produced antihyperglycemic effect, superior weight loss and lipid lowering activity as compared to selective GLP-1 agonist. [18]. In overweight and obese patients native OXM s.c. injection shown to significantly reduce body weight by 1.7 kg in four weeks. OXM was also proven to reduce food intake and energy expenditure in humans [19,20]. A novel single peptide with dual GLP-1/glucagon co-agonism to control hyperglycemia and obesity would be one of the strategies to combat diabesity. Recent research findings toward this direction is very encouraging [8].

It has been demonstrated for decades that acute glucagon administration reduces food intake [21]. In recent years, thermogenic properties of glucagon have been identified. It has been reported that chronic stimulation of glucagon receptor causes lipolysis and body weight reduction [17]. Simultaneous infusion of GLP-1 and glucagon peptide reduced food intake and increase energy expenditure in humans. [22,23]. The rationale for dual GLP-1/glucagon agonism is to combine thermogenic and lipolytic effects of glucagon with the glucose-dependent insulinoetric and anorectic activity of GLP-1 without causing glucagon-associated hyperglycemia to eventually promote substantial weight loss and dramatic glycemic control in diabesity. This rationale is possible because both GLP-1 and glucagon acts via structurally related receptors and both peptide hormones have similar amino acid sequence at N-terminal region. Therefore, it is possible to make single peptide molecule with balanced GLP-1/glucagon receptor agonistic activities. Recently, two independent research groups have published their work in this direction and proved that it is practically possible to make such dual agonist peptide. Day et al. have developed a single peptide using glucagon-based scaffold and incorporated native GLP-1 into it. They made this molecule resistant to proteolytic cleavage by adding rare amino acid 2-aminoisobutyric acid (Aib) at the second position and lactam bridge linker. Day et al., have also added 40 KDa linear polyethylene glycol (PEG) moiety to enhance duration of action. In preclinical studies, PEGylated GLP-1/glucagon dual agonist peptide showed significantly more body weight loss, increased energy expenditure, and improved hepatic steatosis when compared with PEGylated selective GLP-1 agonist in diet-induced obese (DIO) mice [24,8].

Native OXM has a short half-life due to degradation by DPP-IV. Poci et al. have developed DPP-IV resistant GLP-1/glucagon co-agonist peptide using OXM-based structure. They have also incorporated a cholesterol moiety and mini PEG spacer to improve duration of action. GLP-1/glucagon dual agonist produced a significantly more weight loss and reduced food intake as compared to the selective GLP-1 agonist in DIO mice [25]. These independent investigations provide the proof of concept that dual agonists of GLP-1/glucagon have a definite advantage over the mono - agonist and showed a better weight loss as well as glycemic control. It also counterbalances the hyperglycemic liability of glucagon agonist. The relative ratio of GLP-1 receptor co-agonists need to be evaluated carefully for each species to maximize weight loss efficacy and minimize hyperglycemia (Fig. 2). Due to advantage of dual agonist activity over mono-GLP-1 agonist, many biotech and pharmaceutical companies are developing single peptide with dual agonist activity (Table 3).

Safety issues associated with new antidiabetic therapies

In 2007, it has been reported that rosiglitazone, a thiazolidinedione class of drug increased cardiovascular event rate in T2D patients [26]. Cardiovascular events are a major cause of mortality and morbidity in diabetic patients. The antidiabetic therapy directed at improving hyperglycemia might increase the rate of cardiovascular events is a legitimate issue. Therefore, in 2008 U.S. Food and Drug Administration (USFDA) issued guidelines to assess cardiovascular safety in new antidiabetic therapies [27]. Newer GLP-1 RAs are contraindicated in patients with thyroid carcinoma, pan-
cretic cancer, and acute pancreatitis. Gastrointestinal (GI) side effects are also more common with this class of drugs [11]. However, the cardiovascular safety data for the newer agents are limited and on-going clinical trials will reveal the long-term cardiovascular safety of these newer agents. Native glucagon and GLP-1 have positive inotropic and chronotropic activities on the heart. However, it is interesting to note that OXM (GLP-1/glucagon co-agonist) administration showed no increase in heart rate in rat and humans [15]. Therefore, we strongly believe that GLP-1/glucagon co-agonists may have better cardiovascular safety and antidiabetes effects.

Conclusion

Bariatric surgery is the only option to produce a lasting body weight reduction for obese high-risk diabetic patients [28]. However, alterations in physiologically important peptide hormones and cost of the surgery limit their use. Synthetic and metabolically stable peptide represents a potential long-term therapeutic intervention to treat diabetes. Currently available GLP-1 RAs for clinical use have better glycemic control, but body weight reduction is moderate and data vary between different drugs. DPP-4 inhibitors are well tolerated and produce better glycemic control, but are weight neutral. Clinical data with SGLT2 inhibitors are encouraging in terms of cardiovascular safety and body weight reductions. However, Sodium glucose co-transporter 2 inhibitors (SGLT2i) are associated with urinary tract infections and euglycemic diabetic ketoacidosis [29]. It is myth that patients dislike injected therapies. Data suggest that many patients accept injected therapies if they lead to improved outcomes. Currently available GLP-1 RAs are injectables. However, the recent limited literature suggests that oral therapy with peptide-based drugs is possible and there is good progress in the field (Table 1). Novel GLP-1/glucagon co-agonists may have potential to lower hyperglycemia and body weight effectively in the patients suffering from diabetes (Fig. 2). However, it is important that dual agonists should be well balanced to get maximum efficacy and better safety to treat diabetes (Table 3). It’s not far from reality when this class of drugs will be available for diabetes patients and will serve our future generations.

Conflict of interest

None.

References

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