Bioorganic & Medicinal Chemistry Letters 23 (2013) 4011–4018

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

BMCL Digest

Recent progress and future options in the development of GLP-1 receptor agonists for the treatment of diabesity

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ARTICLE INFO

Article history:
Received 16 March 2013
Revised 6 May 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
GLP-1 receptor agonists
Gastro-intestinal hormones
Diabesity
Delivery technologies

ABSTRACT

The dramatic rise of the twin epidemics, type 2 diabetes and obesity is associated with increased mortality and morbidity worldwide. Based on this global development there is clinical need for anti-diabetic therapies with accompanied weight reduction. From the approved therapies, the injectable glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are the only class of agents which are associated with a modest weight reduction. Physiological effects of the gastro-intestinal hormone GLP-1 are improvement of glycemic control as well as a reduction in appetite and food intake. Different approaches are currently under clinical evaluation to optimize the therapeutic potential of GLP-1 RAs directed to once-weekly up to once-monthly administration. The next generation of peptidic co-agonists comprises the activity of GLP-1 plus additional gastro-intestinal hormones with the potential for increased therapeutic benefits compared to GLP-1 RAs.

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Introduction. Type 2 diabetes mellitus (T2DM) is a progressive chronic disease characterized by hyperglycemia due to defective insulin secretion and resistance to insulin action. The disease is associated with morbidity and mortality in patients, and is a leading cause for cardiovascular (CV) disease, renal failure, blindness, amputations and hospitalizations.[1,2] T2DM has recently reached epidemic proportions in both developed and developing countries in conjunction with a substantial increase in obesity.[3] Up to 80% of people with T2DM are overweight or obese, whereas obesity is considered as a major risk factor for T2DM.[4] 'Diabesity' is a new term to characterize this phenomenon of obesity-dependent diabetes associated with multiple comorbidities (mainly CV disease) and in addition describes a rising epidemic.[5] An effective approach to the management of diabesity is a modest reduction in body weight (~4–5 kg) resulting in highly beneficial effects on glycemic control as well as reduced morbidity and mortality.[4] According to the new guidelines of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) out of available classes of glucose-lowering agents, the only class associated with modest body weight reduction are peptidic glucagon-like peptide-1 receptor agonists (GLP-1 RAs). All other agents are either weight neutral (Metformin, DPP4-inhibitors) or associated with weight gain (basal insulin, thiazolidinediones, sulfonylureas).[6]

Therapy with peptidic GLP-1 RAs is based on self-administration by subcutaneous injection using a pen device (or a syringe). The following GLP-1 RAs are approved for the treatment of diabetes: twice-daily exenatide BID (Byetta®, BMS), once-daily lixisenatide (Lyxumia®, Sanofi/Zealand), once-daily liraglutide (Victoza®, Novo Nordisk) and once-weekly exenatide LAR (Bydureon®, BMS).

Current optimization of peptidic GLP-1 RAs aims to improve efficacy, tolerability and compliance such as frequency and ease of administration. Furthermore, significant efforts are currently undertaken to identify therapeutic peptide approaches with the potential for greater body weight reduction compared to existing GLP-1 RAs. The present article reviews key data on marketed and novel long-acting GLP-1 RAs currently in late clinical development, describes novel techniques suitable for once-weekly up to once-monthly administration and discusses potential novel peptide approaches based on further gut hormones. However, recent research activities have resulted also in peptidic and small molecule GLP-1 RAs which might be suited for oral application[7]–among those TTP054 from Transtech Pharma as most advanced in phase 2.[8] Those still early developments in the field of orally available GLP-1 RAs are not covered by this review.

GLP-1 RAs: overview. Natural human glucagon-like peptide-1 (GLP-1) is an incretin hormone derived from the transcription product of the proglucagon gene. It is produced by intestinal L-cells and released in response to meal intake to induce insulin secretion from pancreatic β-cells. The biologically active forms of GLP-1 are GLP-1(7–37) and GLP-1(7–36)NH_2, which exert their action by activation of the GLP-1 receptor, a class B G-protein-coupled receptor. GLP-1 shows a helical character in solution and in the

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http://dx.doi.org/10.1016/j.bmcl.2013.05.022

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receptor-bound conformation (see Fig. 1).[9,10] Exendin-4 is another potent peptidic GLP-1 RA, which was isolated from the venom of the Gila monster. Although it shares only 53% sequence identity with native GLP-1, both peptides show a helical character with many of the conserved amino acids facing the interaction site of the receptor (see Fig. 1).[11] All GLP-1 RAs on the market or in clinical development are either derived from the natural GLP-1 or exendin-4 peptide. Based on their PK-profile, marketed GLP-1 RAs exenatide BID and lixisenatide could be classified as shorter-acting agents compared with longer-acting agents liraglutide and exenatide LAR.

GLP-1 and GLP-1 RAs have 3 major pharmacological activities[12,13] to improve glycemic control in patients with T2DM by reducing fasting and postprandial glucose (FPG and PPG): (i) increased glucose-dependent insulin secretion (improved first- and second-phase), (ii) glucagon suppressing activity under hyperglycemic conditions, (iii) delay of gastric emptying rate resulting in retarded absorption of meal-derived glucose. Treatment with GLP-1 RAs for 12–52 weeks leads to reduction in glycosylated hemoglobin (HbA1c), a diagnostic marker reflecting the average glucose levels of the last 2–3 months (and serving as primary efficacy endpoint in phase 2b–3 studies) of 1.1–1.6% due to reduced postprandial and fasting blood glucose.[14] Unlike insulin or insulin secretagogues (sulfonylureas) the insulinotropic effect of GLP-1 RAs is strictly glucose-dependent resulting in a generally low risk for hypoglycemia. Beyond anti-hyperglycemic actions of GLP-1 RAs, central and possibly peripheral effects (e.g. vagal afferents) which modulate feelings of appetite and satiety are considered to reduce food intake and finally account for a moderate body weight reduction in the range of ~1–3 kg.[15]

In addition, beneficial reduction in systolic and diastolic blood pressure as well as reduced cholesterol plasma levels were observed in clinical trials.[16] Preclinical data illustrate different cardioprotective effects of GLP-1 RAs (e.g. on cardiomyocytes, blood vessels, adipocytes and lipids).[17] These effects might impair the onset or progression of atherosclerotic disease. Furthermore, animal and initial clinical studies have shown that GLP-1 RAs could reduce ischemia–reperfusion injury. However, treatment with longer-acting GLP-1 RAs (liraglutide and exenatide LAR) is accompanied in humans by a slight increase in heart rate of 2–4 beats per minute. Ongoing cardiovascular outcome studies for the marketed agents will reveal if the potential harm of an increased heart rate will be outweighed by the reported cardiovascular benefits.[17]

The most common side effects of GLP-RAs are nausea, vomiting and diarrhea, which are mostly transient and can be at least partly reduced by gradual uptitration of the dose. Beyond gastro-intestinal side effects an association of long-term GLP-1RA therapy with an increased risk for pancreatitis is under discussion. Some rare cases of acute pancreatitis have been reported as postmarketing surveillance activities for GLP-1 RAs. It is not clear if such cases are therapy-related or due to a general 2–3-fold increased risk for acute pancreatitis associated with T2DM.[18] In rodents malignant thyroid C-cell carcinomas were observed following treatment with liraglutide. The human relevance of these findings in rodents could not be determined by clinical or nonclinical studies.[18]

As peptide based drugs, GLP-1 RAs have the potential to induce an immune response and antibody formation was reported for all marketed GLP-1 RAs but does not appear to impact efficacy or safety of these agents. The level of glycemic control (HbA1c) is overall reported similar regardless of the antibody status, although for a rare number of patients high-titer antibody formation might be associated with attenuated glycemic response (reported for 1–4% of total patients treated with exenatide BID).[19] There seems to be slightly increased antibody development and injection site reactions with exendin-4 compared to the GLP-1 analog liraglutide,[20] presumably due to the greater differences in sequence identity compared to native GLP-1.

A differentiating characteristic within the GLP-1 RA class important for glycemic control is the propensity to slow gastric emptying, which is dependent on the pharmacokinetics of these agents. For longer-acting agents such as liraglutide and exenatide LAR, delayed gastric emptying fades following the first dose probably reflecting tachyphylaxis.[21,22] These agents strongly reduce fasting blood glucose probably via continuous increased insulin levels in the fasting state and cause an apparently better efficacy than

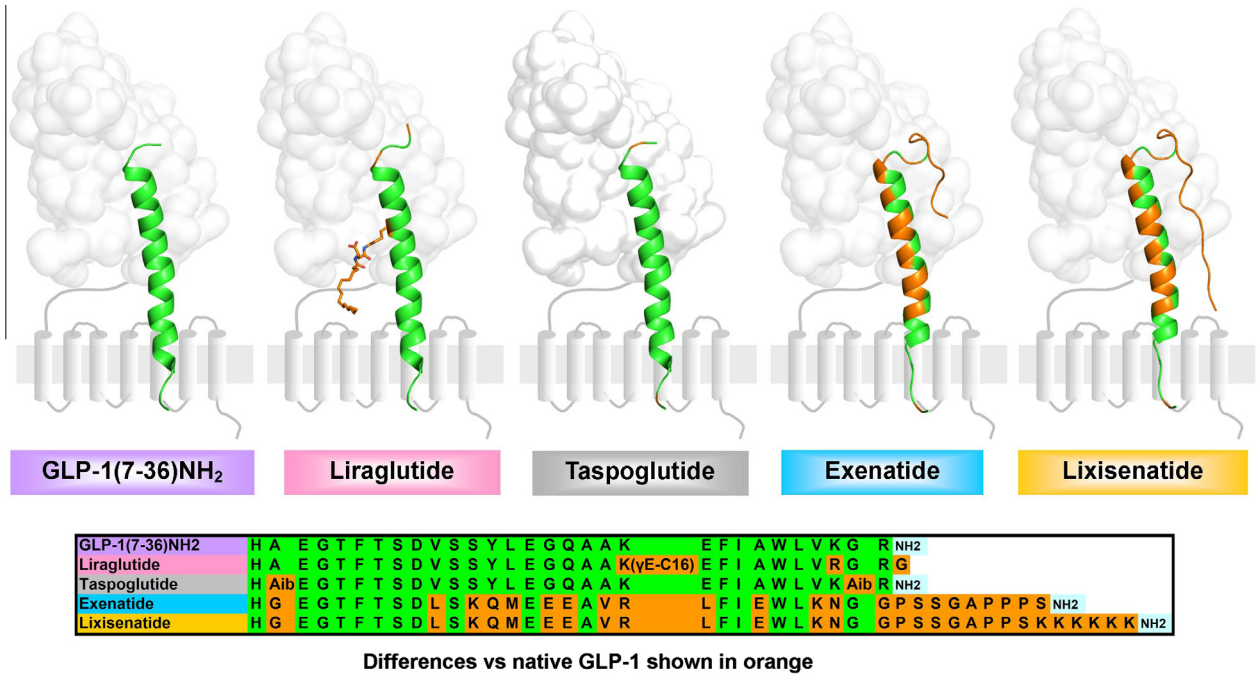

Figure 1. Binding hypotheses of GLP-1 RAs at the GLP-1R. Amino acid changes versus native GLP-1 are shown in orange. Binding mode of GLP-1 from RCSB PDB (PDB ID 3iol), binding modes of the remaining peptides are computational models.

shorter-acting GLP-1 RAs.[14] In contrast, the shorter-acting agents, exenatide BID and lixisenatide are both proven to strongly slow gastric emptying in patients with T2DM without any signs for desensitization with continuous treatment.[23,24] As consequence, shorter-acting GLP-1 RAs are associated with a pronounced reduction in PPG. As HbA1c levels decrease under therapy, the contribution of postprandial glucose to HbA1c increases over that of fasting plasma glucose.[25] For patients with improved HbA1c under therapy (including those on insulin treatment), who have a greater dependency on postprandial glucose, shorter-acting agents may be the better choice. This greater PPG-lowering effect of shorter-acting GLP-1 RAs would be complementary to the glucose lowering effects of basal insulin, which primarily targets fasting glucose. Hence, a combination therapy of shorter-acting GLP-1 RAs with basal insulin may offer the advantage to lower basal insulin requirements and result in beneficial weight loss effects.[26]

GLP-1 agonists on the market and in clinical development. Therapeutic utility of natural GLP-1 is limited by its rapid degradation by serum proteases, predominantly dipeptidyl peptidase IV (DPPIV), but also other enzymes such as neutral endopeptidase (NEP), plasma kallikrein or plasmin.[27] Therefore, GLP-1 has a very short half-life of ~2 min following intravenous administration. A variety of strategies have been applied to provide novel GLP-1 RAs with longer-term in vivo activity following subcutaneous administration, which are described below.

Peptides stabilized against DPPIV by amino acid exchange. One strategy to prolong in vivo half-life is stabilization towards degradation by DPPIV, which preferably cleaves N-terminal Xaa–Pro or Xaa–Ala dipeptide sequences. Alteration of that N-terminal sequence, especially the second amino acid, has proven to reduce degradation by DPPIV.[28,29]

Exenatide BID. In exendin-4, the second amino acid is a Gly rendering it resistant to DPPIV mediated degradation. Furthermore, the Leu21–Ser39 span of exendin-4 forms a compact tertiary fold (the Trp-cage) which shields the side chain of Trp25 from solvent exposure, leading to enhanced helicity and stability of the peptide (see Fig. 1).[9] Exenatide BID (Amylin, now BMS), a synthetic version of exendin-4, represents the first GLP-1 RA approved in 2005 as antidiabetic therapy for the treatment of T2DM. It has a terminal half-life of ~2.4 h after subcutaneous administration and is applied twice daily (10 μg). With this dosing regimen as monotherapy or in combination with oral antidiabetics, HbA1c reductions of 0.8–0.9% are typically observed accompanied by a body weight reduction in the range of 1.8–2.6 kg following a treatment duration of 30 weeks. In an open label extension the combination of exenatide BID and metformin showed a HbA1c reduction of 1.1% and a body weight reduction of 4.4 kg after 82 weeks.[30] Exenatide BID and insulin glargine did not demonstrate a significant difference in HbA1c reduction (1.25% vs 1.26%) after 26 weeks treatment. However, insulin glargine resulted in a weight gain of 3 kg compared to a reduction of 2.7 kg seen with exenatide BID.[31]

Lixisenatide. Lixisenatide, developed by Sanofi/Zealand, is a synthetic analog of exendin-4 (Fig. 1). Compared to exendin-4, six Lys residues have been added to the C-terminus (also amidated), while one Pro in the C-terminal region has been deleted. Lixisenatide has a mean terminal half-life of approximately 3 h in humans. In a dose-ranging study in patients with T2DM, inadequately controlled with metformin, lixisenatide at a dose of 20 μg once-daily demonstrated the best efficacy-to-tolerability ratio.[32] Lixisenatide was applied as once-daily administration (20 μg) in phase 3 trials and has demonstrated efficacy as monotherapy, in combination with oral antidiabetic drugs, and as add-on to basal insulin, with particular efficacy in reducing postprandial glucose excursion (HbA1c reduction up to 0.9%). In combination with oral antidiabetic drugs, lixisenatide resulted in a sustained body weight reduction from baseline in controlled studies (24 weeks) in a range from 1.8–3 kg. Comparison of lixisenatide with exenatide BID as add-on to metformin demonstrated noninferior improvements in HbA1c, but with less hypoglycemia, slightly less weight loss and a more favorable gastro-intestinal tolerability profile after 24 weeks.[33] Lixisenatide significantly delays gastric emptying, a process which is accompanied by strong post-prandial glucose lowering.[34] These attributes are very attractive with regard to a combination therapy with basal insulins, for example, insulin glargine, where Lixisenatide demonstrated significantly reduced HbA1C (0.7–0.8%) and body weight (1.8 kg).[34] In February 2013, the EMA granted marketing authorization in Europe for lixisenatide for the treatment of adults with T2DM.[35]

Sustained release of DPPIV stabilized peptides. DPPIV-resistant peptidic GLP-1 RAs are still subjected to renal clearance as illustrated by the rather moderate half-lives of exenatide and lixisenatide. Therefore, several approaches have been explored to further prolong the duration of action by either creating a depot (e.g. in the subcutaneous space), from which the peptide is slowly released, or by conjugating peptidic GLP-1 agonists to carrier molecules in order to reduce renal clearance.

Taspoglutide. Taspoglutide developed by Roche (in collaboration with Ipsen) is a close analog of natural GLP-1(7–36) in which the unnatural amino acid aminoisobutyric acid (Aib) has been introduced in position 8 and 35 in order to avoid degradation by DPPIV, but also by other serine proteases such as plasma kallikrein and plasmin.[36] NMR-studies with taspoglutide showed a similar secondary structure compared to native GLP-1, but clearly an increased α-helicity in the C-terminal part of the peptide. Taspoglutide was developed as a sustained release formulation containing zinc chloride suitable for once-weekly administration. When injected into the subcutaneous tissue, taspoglutide precipitates and forms a depot. Taspoglutide was studied in several phase 3 studies with a 10 and 20 mg once-weekly dosing regimen, and showed consistent HbA1c and weight reductions.[37–41] Significant weight reductions were only observed with the 20 mg dose.[42] Once-weekly taspoglutide was superior to twice-daily exenatide with respect to glycemic control, while resulting in a similar weight loss after treatment for 24 and 52 weeks. The overall safety profile comprising gastro-intestinal tolerability, systemic allergic reactions and injection-site reactions was clearly worse, especially the gastro-intestinal side effects, which resulted in a roughly doubled discontinuation rate in patients treated with taspoglutide compared to exenatide (34 vs 16%). Based on this phase 3 data Roche decided to stop the development of taspoglutide.[43]

Exenatide LAR–Bydureon. Further strategies to create a subcutaneous depot from which the peptidic GLP-1 RA of choice can be slowly released utilize polymeric matrices. Exenatide LAR (developed by Amylin/Lilly/Alkermes–now BMS) is a once-weekly formulation of exenatide, in which exenatide is noncovalently entrapped into a biodegradable polymeric matrix consisting of poly(D,L-lactide-co-glycolide) (PLG) forming microspheres.[44] Slow release from the polymeric matrix takes place through diffusion and microsphere breakdown. Exenatide LAR has a half-life in humans of 5–6 days. After a 2 mg s.c. injection, steady state plasma levels of exendin-4 are typically obtained after 6–10 weeks.[13] At that dose HbA1c reductions of 1.3–1.9% were observed. A direct comparison with exenatide BID revealed a better reduction in HbA1c (1.9 vs 1.5%) with a similar reduction in body weight (3.6 vs 3.7 kg).[45] Different from this efficacy data, a head-to-head comparison with liraglutide showed an improved glycemic control following 26 weeks of treatment associated with increased weight reduction in favor of liraglutide (HbA1c reduction 1.5% vs 1.3%, body weight reduction 3.6 kg vs 2.7 kg).[46] One drawback of exenatide LAR is the relatively large needle size (23 gauge), which is used for administration due to the viscosity of the polymeric suspension, as well as the quite laborious preparation prior to injection.

Fatty acid conjugation. The concept of fatty acid conjugation is a well established strategy to prolong the action of peptides by facilitating binding to serum albumin thereby reducing their renal clearance. Landmark achievements in this area have been the development of long acting insulin analogs.[47,48]

Liraglutide. Liraglutide (Victoza®) developed by Novo Nordisk is a close structural homolog to GLP-1(7–37) with 97% sequence identity to the native hormone. Lys in position 34 is substituted by Arg and a palmitic acid is conjugated to Lys in position 26 via a glutamate spacer (see Fig. 2a).[20] This combination of spacer and fatty acid length turned out to be optimal in terms of in vitro activity and in vivo prolonged duration of action in pigs.[49,50] The mechanisms for prolongation of action are manifold. Following subcutaneous injection, the peptide is slowly released from the injection site due to self-association.[51] Once it enters the bloodstream, liraglutide is extensively bound to serum albumin (~99%), which leads to an increased enzymatic stability towards DPPIV and NEP while reducing renal clearance. The plasma half-life in humans is 11–13 h.[20] Liraglutide was approved in 2009 for the treatment of T2DM. The therapeutic standard dose is 1.2 mg once-daily with possible increase to 1.8 mg to further improve glycemic control. The HbA1c reductions observed with these dosing regimens in phase 3 trials were 1.1–1.8% accompanied by a weight loss of around 2–3 kg (26 week treatment).[52] In a direct comparison liraglutide once-daily showed significant improvements in glycemic control compared with *exenatide* twice-daily (HbA1c reduction of 1.12% vs 0.79%).[53] Using higher doses (3 mg) liraglutide is also under development (phase 3) for the treatment of obesity[54] as well as of obese patients with T2DM.[55] In a phase 3a study liraglutide 3 mg treatment resulted in ~6% weight reduction in overweight to obese people compared to placebo.[56] Among the class of GLP-1 RAs, liraglutide currently seems to be the most effective agent for the treatment of obesity.

Semaglutide. Semaglutide is a next generation GLP-1 analog from Novo Nordisk, currently in phase 3 clinical development for T2DM as a once-weekly injection. Semaglutide's structure is based on liraglutide, with two further modifications: Gly in position 8 is replaced by Aib, a beneficial modification already applied in taspoglutide. Furthermore, the fatty acid side chain has been modified towards a *N*6-[*N*-(17-carboxy-1-oxoheptadecyl-L-γ-glutamyl[2-(2-aminoethoxy)ethoxy]acetyl[2-(2-aminoethoxy)ethoxy]acetyl] residue (see Fig. 2a). The human half-life is ~160 h. In a 12 week phase 2 trial in T2DM patients, semaglutide was tested at 5 doses (0.1–1.6 mg) once-weekly. Semaglutide ⩾0.2 mg dose dependently reduced HbA1c from baseline up to 1.7% (vs 0.5% reduction for placebo) and for doses ⩾0.8 mg also body weight by up to 4.8 kg (vs 1.2 kg reduction for placebo).[57]

Conjugation to albumin. Another half-life prolonging principle is the (genetic) fusion to recombinant albumin. Human serum albumin (HSA) has a molecular weight of ~67 kDa.[58] The half-life of albumin in humans is ~19 days. PH-dependent recycling mediated by the neonatal FC receptor (FcRN) has been shown to contribute to that long half-life.[59] When albumin is fused to therapeutic peptides such as GLP-1 RAs, both FcRN mediated recycling as well as reduced clearance due to the increased molecular weight are responsible for half-life prolongation.

Albiglutide. In albiglutide, developed by GlaxoSmithKline (GSK), two copies of GLP-1 are fused as tandem repeat to the N-terminus of albumin. DPPIV-resistance is achieved by a single substitution, Ala for Gly, at the DPPIV cleavage site (see Fig. 2b). The tandem repeat unit was developed to overcome reduced potency as was seen when only one GLP-1 moiety was directly fused to albumin, meaning that one GLP-1 moiety of the tandem repeat serves as a spacer to allow potent binding to the GLP-1 receptor.[60] Albiglutide has a half-life of 6–8 days in humans and is currently in phase 3 for the treatment of T2DM as once-weekly injection. In a 32-week head-to-head study comparing albiglutide (50 mg) to once-daily liraglutide (1.8 mg) (HARMONY 7), albiglutide demonstrated a statistically significant reduction in HbA1c (0.78%) from baseline.[61] The rather moderate body weight loss of ~0.6 kg seen for

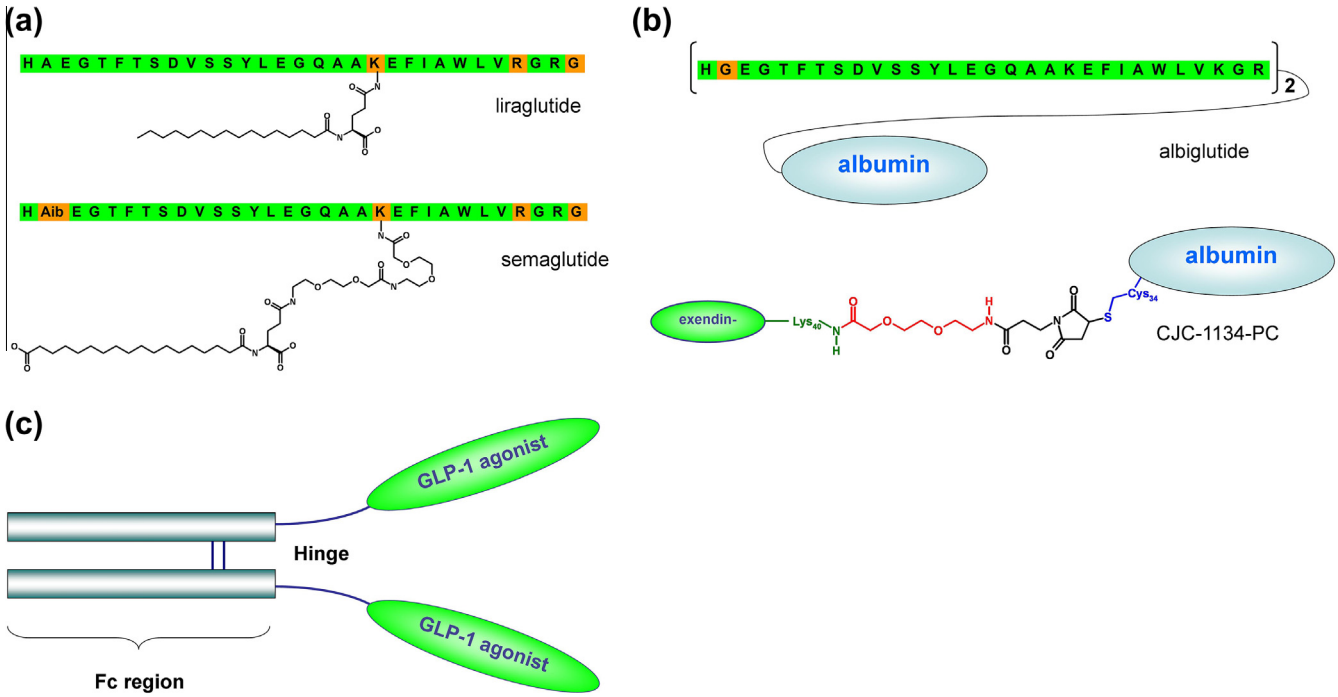

Figure 2. Approaches for half-life extension of GLP-1 agonists. (a) Liraglutide and semaglutide carry fatty acids, which facilitate binding to serum albumin thereby reducing their renal clearance. (b) In albiglutide, two copies of modified GLP-1 are fused as tandem repeat to the N-terminus of albumin. (c) Similar to albumin fusion, peptides can be linked to the Fc region of immunoglobulin G (IgG) as applied in dulaglutide.

albiglutide is thought to be linked to limited actions in the CNS due to its large molecular size.[13] A regulatory application for albiglutide was submitted in the US in January 2013.[62]

CJC-1134-PC. Likewise, in ConjuChem's CJC-1134-PC, exendin-4 is coupled via its C-terminus to albumin by a chemical linker bearing a terminal maleimide which is used for the chemical conjugation to a single cystein residue on albumin (see Fig. 2b).[63] CJC-1134-PC has a half-life of ~8 days in humans and is currently in phase 2 trials for the treatment of T2DM as once-weekly injection. CJC-1134-PC seems to be more potent compared to albiglutide. In phase 2 trials the once-weekly application of 2 mg led to a HbA1c reduction of 1.4%. As observed for albiglutide, the effects on weight were only moderate.[64]

FC fusion. Similar to albumin fusion, peptides can be linked to the constant region of immunoglobulin G (IgG), the Fc region (see Fig. 2c).[65] The Fc region of IgG has a half-life of ~22 days.[66] Likewise, when fusing peptides to the FC region of IgG the protraction principle is based on reduced renal clearance and FcRN mediated receptor recycling.

Dulaglutide. Dulaglutide (Eli Lilly) is a recombinant fusion protein, which consists of two GLP-1 peptides covalently linked by a small peptide [tetraglycyl-L-seryltetraglycyl-L-seryltetraglycyl-L-seryl-L-alanyl] to a human IgG4-Fc heavy chain variant. Compared to natural GLP-1, the GLP-1 moieties contain amino acid substitutions (Ala8→Gly, Gly26→Glu, Arg36→Gly) to ensure protection from DPPIV cleavage as well as maintenance of the potency of the construct. Dulaglutide has a half-life of ~4 days and is in advanced clinical trials as once-weekly injection for the treatment of T2DM.[67] In phase 2 clinical trials dulaglutide showed significant dose-dependent reduction in HbA1c (1.5% for the 1.5 mg dose after 12 weeks) and dose-dependent reductions in body weight. However, the decrease in body weight was not statistically significant when compared to the placebo group.[68] Currently, a large phase 3 program (AWARD studies Nos. 1–7) is ongoing, in which dulaglutide has shown a superior glycemic control compared to exenatide BID after 6 month of treatment.[69] Detailed reports regarding the efficacy in weight loss in longer studies are not yet available.

Langlenatide (*HM11260C*). In langlenatide, developed by Hanmi Pharmaceuticals, an exendin-4 analog is fused via a short polyethyleneglycol (PEG) linker to a nonglycosylated Fc region ('LAPS-carrier'). In contrast to dulaglutide only one peptide copy is fused to the Fc-carrier. Such monomeric peptide-Fc-fusion constructs hold promise to show even higher in vivo efficacy and longer duration of action. In fact, the half-life of langlenatide has been reported to be ~150 h.[70] Langlenatide is currently being investigated in phase 2 trials for the treatment of T2DM as once-weekly injection (1–4 mg doses), but also for its suitability as once-monthly application (8–16 mg).[71]

Peptides coupled to biological polymers–Xtenylation. Xtenylation, which is the genetic fusion of an unstructured recombinant polypeptide to a peptide or protein, is a further generic approach to extend plasma half-life of peptides, again by reducing renal clearance. The Xten protein is a nonrepetitive amino acid polymer, which comprises 864 amino acids selected from Ser, Ala, Pro, Thr, Glu and Gly, with a huge hydrodynamic volume and good serum stability.[72] In contrast to polyethyleneglycol (PEG), Xten is highly biodegradable which might be beneficial with respect to safety.

VRS-859: In VRS-859, Xten is genetically fused to exenatide. The product shows a half-life in cynomolgous monkeys of ~3 days. VRS-859 is currently being developed by Diartis Pharmaceuticals as once-monthly treatment for patients with T2DM. In October 2012, phase 1 results in T2DM patients were disclosed showing statistically significant reductions in HbA1c levels at 30 days after only one 200 mg dose of VRS-859, supporting the once-monthly dosing regimen.[73]

Novel approaches and combination of multiple hormone actions. Approximately 70–80% of severely obese patients with T2DM who undergo bariatric surgery show significant and fast improvement of glycemic control even up to a complete remission of diabetes, often resulting in discontinuation of insulin or oral antidiabetic therapy.[74] The mechanism seems partly independent of weight reduction and related to alterations in the release of gastro-intestinal hormones, for example increased plasma levels of GLP-1, oxyntomodulin and peptide YY (PYY). In addition to the effects of GLP-1, these peptides are known to be involved in the regulation of glucose homeostasis, food intake and energy expenditure.[75] Based on the impressive improvements of T2DM after bariatric surgery, trying to mimic some alterations in plasma levels of these peptide hormones is a highly attractive research effort to identify novel therapeutic options for obese patients with T2DM. Combining dual or triple agonistic effects in a single peptide (feasible if peptides are sufficiently similar) or combining 2 distinct peptides in a formulation approach are possible options. Slow release formulations/delivery approaches as mentioned above could prolong the duration of action from once-daily up to a once-weekly administration regimens.

Below we discuss promising combinations of peptides, which have the potential for improved glycemic control associated with more effective weight reduction compared to pure GLP-1 RAs.

GLP-1/glucagon co-agonists. Oxyntomodulin is a 37 amino acid peptide (comprising the entire 29 amino acid sequence from glucagon plus a C-terminal extension of 8 residues) secreted by intestinal L-cells (together with GLP-1 and PYY) following meal ingestion. The dual agonistic activity of oxyntomodulin is weaker for the GLP-1 and glucagon receptors when compared to the cognate native ligands GLP-1 and glucagon.[76] Originally glucagon was discovered as a hormone with effects counter to those of insulin, raising blood glucose levels by stimulating gluconeogenesis and glycogenolysis to circumvent a hypoglycemic state. However, more recent data in rodents and humans reveal that glucagon could have beneficial effects on energy balance, body fat mass and nutrient intake. Those effects seem to be mediated at least in part by FGF21-dependent pathways.[77,78]

In overweight and obese people native oxyntomodulin was shown to significantly reduce body weight by ~1.7 kg vs. placebo following three-times subcutaneous administration (to compensate for the short half-life of the native peptide) for 4 weeks.[79] Furthermore, oxyntomodulin was mechanistically proven to reduce food intake after an ad libitum test meal and increase energy expenditure in humans.[80] Thus, combining the actions of GLP-1 and glucagon in one molecule like in oxyntomodulin might lead to a therapeutic principle with anti-diabetic action related to the GLP-1 component and a pronounced weight lowering effect related to glucagon receptor stimulation. Pioneering work in the field was carried out by Bloom and co-workers,[81] DiMarchi and co-workers[82] and Merck Research Laboratories.[83] Chemical strategies in order to identify such GLP-1/glucagon co-agonists started either from the sequence of oxyntomodulin or glucagon by carefully incorporating amino acids known to be relevant for GLP-1 agonism but also elements to prolong half-life. A Tschöp, model peptide is shown in Figure 3, which is a hybrid peptide derived from native GLP-1 and glucagon. Further chemical modifications to increase the stability and in vivo half-life include a lactam bond between the side-chains of Glu in position 16 and Lys in position 20 ('stapling') and the addition of a linear 40 kDa PEG chain to Cys in position 24. For the design of novel stabilized peptides the selection of the activity-ratio for the GLP-1 and the glucagon receptor is important for adjusting the right balance between anti-diabetic and anti-obesity effects. High potency at the glucagon receptor can be anticipated to strongly increase weight loss but potentially at the expense of elevated glucose levels. Data in mice suggest that a balanced ratio of GLP-1/glucagon-receptor activation (~1:1) is optimal to ensure glucose control associated with improved

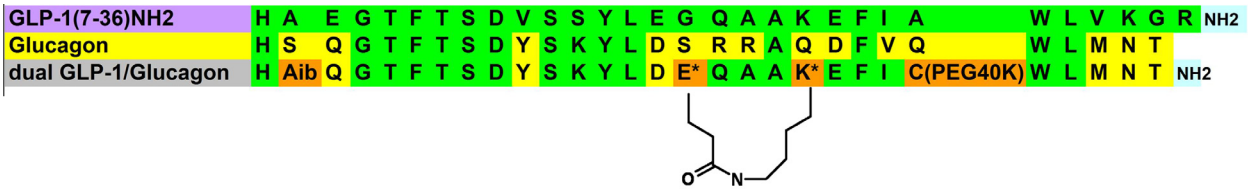

Figure 3. Amino acid sequence of native GLP-1, native glucagon and a representative chimeric peptide[82] which shows balanced activity on both, the GLP-1 and glucagon receptor. Amino acids, which are identical to GLP-1 are colored green, residues unique to glucagon are shown in yellow and further modifications are shown in orange. These modifications include a lactam bond between Glu in position 16 and Lys in position 20 ('stapling') and the addition of a linear 40 kDa PEG chain to Cys in position 24.

weight loss.[84] Whether this concept translates to the human situation has to be proven in clinical settings. A number of compounds have progressed into phase 1, among those ZP2929, which is a GLP-1/Glucagon co-agonist carrying a fatty acid for once-daily dosing from Zealand Pharma (now in collaboration with Boehringer Ingelheim),[85] and TT401 from Transition Therapeutics, which is a PEGylated analog for once-weekly dosing.[86]

GLP-1/GIP co-agonists. The incretin effect refers to enhanced insulin secretion following an oral glucose load relative to an isoglycemic challenge after intravenous administration and is caused by the two incretin hormones, GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). The gut-derived peptide hormone GIP is released by gastro-intestinal K-cells following meal ingestion. GIP shares 37% amino acid sequence identity with GLP-1 and shows a helical character with many conserved or similar amino acids facing the binding groove of the extracellular domain of the GIP receptor that is structurally similar to the GLP-1 receptor.[10,87] According to the similarity of the interaction sites, designing single peptides with activity for both incretin hormone receptors is feasible. Since both hormones account equally for ~50–70% of the overall meal stimulated insulin secretion in humans,[88] a more pronounced anti-diabetic effect of such dual agonistic peptides compared to sole GLP-1 RAs could be expected. Preclinical data for potent GLP-1/GIP dual agonistic peptides show significantly better reduction of blood glucose levels and body weight in diet-induced obesity (DIO) mice compared to liraglutide following once-daily treatment for one week.[89] Furthermore, such a GLP-1/GIP co-agonist was also shown to be superior to liraglutide with respect to glycemic control in a clamp study in nonhuman primates.[90]

Of note, in patients with T2DM the incretin effect is generally impaired and this is considered to be mainly related to a decreased GIP-activity of the β-cell.[91] On that basis, a prerequisite for considering dual GLP-1/GIP receptor agonists as therapeutic option for T2DM is the reversibility of the reduced response of the β-cell to GIP. Indeed, studies in patients with T2DM indicate an amelioration of the insulinotropic effect of GIP when the diabetic status is improved, for example, following moderate life-style induced weight loss of ~5 kg, 4 weeks of intensive insulin treatment or therapy with sulfonylureas.[92–94] Furthermore, there is preclinical evidence in Zucker diabetic rats that the reduced insulinotropic effect of GIP is related to down-regulation of the respective receptor. Two weeks treatment with phlorizin (an unspecific SGLT 1/2-inhibitor) could reestablish receptor expression, which is associated with GIP-induced insulin secretion not observed in the placebo group that was not treated with phlorizin.[95] Marcadia, which was acquired by Roche in late 2010, was pioneering this approach. A first PEGylated compound, Mar701 (RO-6807952), was evaluated in phase 1 clinical settings, among those a 8 weeks study in patients with T2DM on top of metformin. However, that compound was discontinued in September 2012 and replaced by a follow-up compound MAR709 (RG7697/RO6811135), now undergoing phase 1 evaluation.[96,97]

GLP-1/PYY Combination. Peptide YY (PYY), named by the Tyr (Y) residues at each end of its 36 amino acid chain, is a further gut-derived hormone, which is also postprandially secreted from intestinal L-cells into the blood circulation. Following rapid cleavage by DPPIV, PYY(3–36) is generated in plasma, which is a selective agonist for the neuropeptide Y2 receptor. PYY(3–36) was proven to inhibit food intake in humans and showed improved insulin sensitivity in rodents. Furthermore, PYY deficiency is discussed to contribute to the pathogenesis of obesity.[98,99] Along these lines, a combination approach of GLP-1 and PYY seems an attractive option for the treatment of diabesity. Because GLP-1 and PYY lack sufficient similarity, both peptides need to be co-administered. This concept is supported by preclinical data from GSK studying the combination of exendin-4 and PYY constructs fused to an albumin-binding antibody fragment. Following subcutaneous administration every second day over a period of 15 days to diabetic mice (db/db), HbA1c as well as body weight were dramatically reduced suggesting a synergistic effect compared to treatment with each single agent.[100]

Conclusion and outlook. Life-style changes comprising diet and exercise are the first recommendations for patients newly diagnosed with T2DM in order to reduce body weight and thereby to reduce hyperglycemia and decrease the associated cardiovascular risk.[5] This recommendation underlines the importance of weight loss in T2DM especially since prevalence and incidence of T2DM in conjunction with rising obesity rates are increasing worldwide. Thus, there is a high medical need for novel anti-diabetic agents associated with effective weight lowering properties. The marketed GLP-1 RAs have proven successfully such twofold therapeutic benefit by reducing hyperglycemia and body weight to meet this need. Upcoming phase 3 data from the next generation of long-term GLP-1 RAs will show if the efficacy within the class can be further improved without impacting safety. A third wave of therapeutic peptides with combined anti-diabetic and anti-obesity effects are peptidic co-agonists comprising the activity of GLP-1 plus additional gastro-intestinal hormones. Single peptides with dual or even trigonal agonistic activity as well as combinations of two separate peptides with different, but additive effects might have the potential for increased efficacy, especially with respect to body weight reduction, over the class of pure GLP-1 RAs.

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