The Review of DIABETIC STUDIES

REVIEW

New Therapeutic Strategies for the Treatment of Type 2 Diabetes Mellitus Based on Incretins

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■ Abstract

Orally ingested glucose leads to a greater insulin response compared to intravenously administered glucose leading to identical postprandial plasma glucose excursions, a phenomenon referred to as the "incretin effect". The incretin effect comprises up to 60% of the postprandial insulin secretion and is diminished in type 2 diabetes. One of the very important gastrointestinal hormones promoting this effect is glucagon-like peptide 1 (GLP-1). It only stimulates insulin secretion and normalizes blood glucose in humans under hyperglycemic conditions, therefore it does not cause hypoglycemia. Other important physiological actions of GLP-1 are the inhibition of glucagon secretion and gastric emptying. It further acts as a neurotransmitter in the hypothalamus stimulating satiety. *In vitro* and animal data demonstrated that GLP-1 increases β-cell mass by stimulating islet cell neogenesis and by inhibiting apoptosis of islets. In humans, the improvement of β-cell function can be indirectly observed from the increased insulin secretory capacity after GLP-1 infusions. GLP-1 represents an attractive therapeutic principle for type 2 diabetes. However, native GLP-1 is degraded rapidly upon exogenous administration and is therefore not feasible for routine therapy. The first long-acting GLP-1 analog ("incretin mimetic") Exenatide (Byetta®) has just been approved for type 2 diabetes therapy. Other compounds are being investigated in clinical trials (e.g. liraglutide®, CJC1131®). Dipeptidyl-peptidase IV inhibitors (DPP-IV inhibitors; e.g. Vildagliptin®, Sitagliptin®) that inhibit the enzyme responsible for incretin degradation are also under study.

Keywords: type 2 diabetes · incretin · incretin mimetics · GLP-1 · DPP-IV inhibitors · vildagliptin · sitagliptin

Gastrointestinal hormones and the incretin effect

Zunz and La Barre hypothesized as early as 1929 that gastrointestinal factors stimulate insulin secretion after a meal [1]. Physiological studies in the second half of the 20th century showed then that orally administered glucose evoked a greater insulin response than an intravenously administered glucose infusion calculated to lead to exactly the same serum glucose excursions. This difference in the insulin response was named the "incretin effect" and the gastrointestinal hormones stimulating insulin secretion after oral glucose ingestion were called "incretins" [2-4]. The metabolic, neural and hormonal effects of the small intestine on the endocrine pancreas are referred to as "entero-insular axis" (Figure 1). Approximately 30 - 60% of the C-peptide and 80 - 90% of the insulin response after an oral glucose load are conveyed by incretin hormones in non-diabetic subjects, depending on the amount of glucose. In type 2 diabetes, the incretin effect is reduced or even absent [3, 5].

Glucose-dependent insulinotropic peptide (GIP) (also referred to as gastric inhibitory polypeptide) se-

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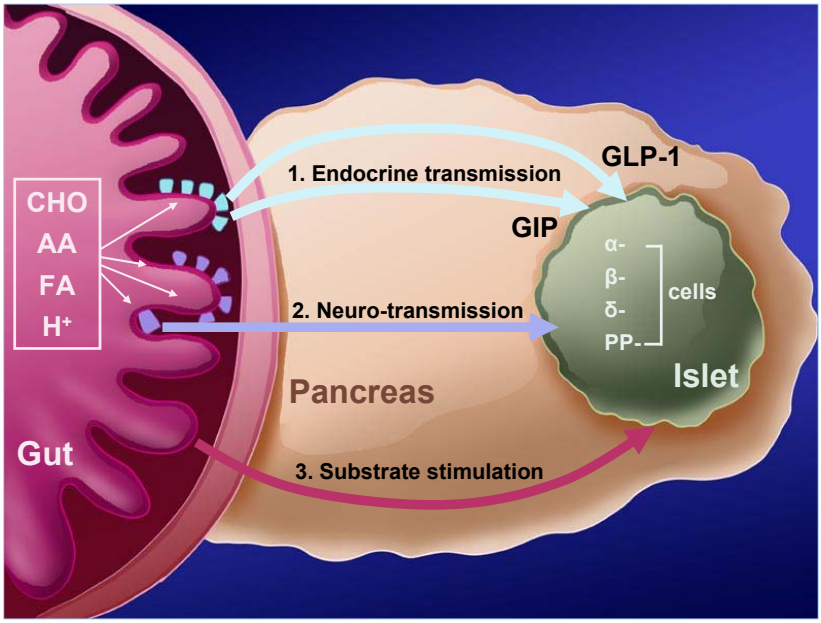

Figure 1. The Enteroinsular Axis. Postprandially, insulin secretion is directly stimulated by substrates, neuro-transmission (through entero-pancreatic nerves activated by chymus and intestinal distension), and by strong endocrine stimulation through incretin hormones. CHO: carbohydrates. AA: amino acids. FA: fatty acids. H^+: hydrogen from gastric acid production. α-cells produce glucagon, β-cells insulin, δ-cells somatostatin, and PP-cells pancreatic polypeptide. (Modified according to [2]).

creted from the entero-endocrine K-cells in the jejunum was the first incretin hormone discovered in 1971, which accounts for approximately 60% of the total incretin effect [4, 6]. In 1985, another important incretin hormone, glucagon-like peptide 1 (GLP-1) was discovered and shortly thereafter found to be a physiological incretin in humans [4, 7, 8].

GLP-1 is a product of the proglucagon gene. It is generated by tissue-specific post-translational processing of proglucagon. The gene is expressed in the A-cells of the pancreas, the neuroendocrine L-cells of the small intestine and the hypothalamus. Biologically active peptides are formed by post-translational cleavage of proglucagon in the previously specified tissues. GLP-1 is mainly formed in the small intestine, whereas glucagon is the major product of the proglucagon processing in the pancreas [8]. GLP-1 is recognized as a potentiator of glucose-induced insulin secretion and substantially contributes to the incretin effect. Plasma concentrations of GLP-1 increase six- to eight-fold after a carbohydrate meal [7]. The physiological relevance of GLP-1 and GIP as incretin hormones is further emphasized by studies with knockout mice which are lacking the GIP or GLP-1 receptor [9, 10].

GLP-1 and the incretin effect in type 2 diabetes

Interestingly, of the two important incretins, GIP has lost most of its insulinotropic potency in type 2 diabetic patients [11, 12]. In contrast, GLP-1 still effectively stimulates insulin secretion in these patients [13-15]. The underlying cause for the diverging properties of GIP and GLP-1 regarding the altered incretin effect in type 2 diabetes is not completely understood. The promising therapeutic potential of GLP-1 as a pharmacological tool for treating type 2 diabetes was proposed in the 1990s, along with the further characterization of the incretin effect [15, 16]. In contrast to other insulinotropic agents, e.g. the sulfonylureas, the insulinotropic effect of GLP-1 depends even more closely on the actual glucose concentration providing the possibility of glucose normalization without the risk of hypoglycemias [15, 17]. As well as the glucose lowering effect via the stimulation of insulin secretion, GLP-1 has a variety of additional physiological effects that may be advantageous in type 2 diabetes therapy.

GLP-1 and its effect on glucagon secretion

GLP-1 inhibits glucagon secretion *in vitro* and *in vivo* [18, 19]. In type 2 diabetes, excessive glucagon secretion in relation to the plasma glucose stimulates hepatic glycogenolysis and therefore contributes to fasting hyperglycemia [20]. In type 2 diabetic patients, infusions of GLP-1 lead to a significant suppression of glucagon secretion together with a normalization in fasting plasma glucose [15]. GLP-1 administration however, does not impair the glucagon counter-regulatory response to hypoglycemia, since the glucagon secretion is glucose-dependent [21].

GLP-1 and the regulation of satiety – central effects in the hypothalamus and peripheral effects in delaying gastric emptying

In the central nervous system, GLP-1 acts as a neurotransmitter in the hypothalamus and stimulates satiety directly. Intraceribroventricular GLP-1 application lead to decreased food intake in rodents [22]. Peripher-

ally, various gastrointestinal functions are influenced directly and indirectly by GLP-1. Gastric emptying after meals is slowed, in addition GLP-1 inhibits gastric acid secretion [23]. In humans, a continuous subcutaneous infusion of GLP-1 over six weeks lead to significant weight loss due to reduced calorie intake attributable to increased feelings of satiety [22, 24]. Whether the effects of GLP-1 on satiety in humans are mainly mediated by the retardation of gastric emptying through a feedback loop or are centrally mediated is still under investigation [25, 26].

GLP-1 and its effect on β-cell mass and function

Experiments in rodent models as well as *in vitro* studies demonstrate an increase of β-cell mass after long-term administration of GLP-1 due to a stimulation of islet cell neogenesis [27-29] from precursor cells on the one hand, and due to an inhibition of apoptosis of β-cells on the other [28, 30]. The restoration of some β-cell function parameters can be detected indirectly from the increased insulin secretory capacity in humans receiving GLP-1. However, an increase of β-cell mass cannot be directly quantified without invasive surgical techniques in humans [31]. In isolated human islets, glucose-dependent insulin secretion and islet cell morphology is significantly improved, when the islets are incubated with GLP-1 [32]. The reason for the expansion of β-cell mass by GLP-1 is due to the inhibition of apoptotic signaling pathways and the stimulation of signaling pathways leading to a proliferation of β-cells [28, 30, 32]. The mRNA levels for Bcl-2 and caspase 3 as markers for apoptotic activity are down regulated in human islets incubated with GLP-1 [32].

GLP-1 as a potential therapeutic principle in the treatment of type 2 diabetes

The incretin effect is known to be reduced in patients with type 2 diabetes, resulting in inappropriately low insulin secretion following oral ingestion of nutrients [33]. More recent studies have indicated that GLP-1 secretion is also impaired in these subjects, suggesting that a reduced meal-related GLP-1 response may contribute to the decreased incretin effect [34]. GLP-1 is effective in patients with type 2 diabetes, increasing insulin secretion and normalizing both fasting and postprandial blood glucose when given as a continuous intravenous infusion (Figure 2) [15], even in subjects with advanced type 2 diabetes long after sulfonylurea secondary failure [35]. Unexpectedly, the effects of a single subcutaneous injection of GLP-1 were disappointing. Although high plasma levels of immunoreactive GLP-1 were achieved, insulin secretion rapidly returned to pre-treatment values and blood glucose concentrations were not normalized [36]. Nevertheless, the effect of repeated subcutaneous administration on fasting blood glucose is as good as that of intravenous administration [36], while continuous subcutaneous administration for 6 weeks reduces fasting and postprandial glucose concentrations and lowers HbA1c concentrations [24].

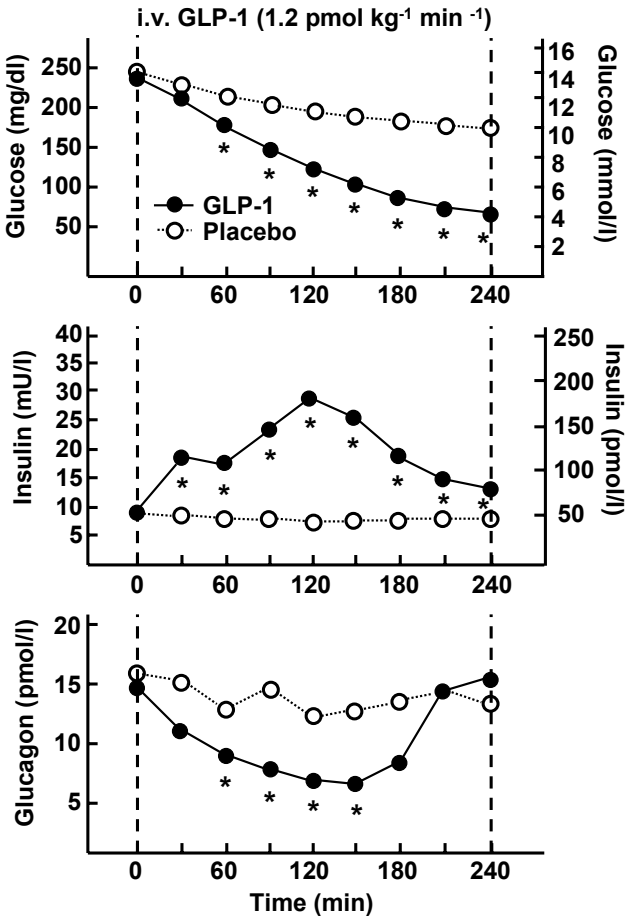

Figure 2. The figure represents plasma glucose, insulin, and glucagon levels after intravenous administration of 1.2 pmol kg^{-1} min^{-1} GLP-1 in 10 type 2 diabetic patients. The time interval of continued GLP-1 infusion (●) and placebo administration (○) was 240 min. Asterisk (*) indicates a significance level of $p < 0.0001$ (modified according to [15]).

The multiple actions of GLP-1 may constitute a new and attractive therapeutic principle for the treatment of type 2 diabetes by improving the postprandial metabolic situation and eliminating hypoglycemic events (Table 1) [24]. The risk of hypoglycemia ob-

served in patients treated with GLP-1 is minimal because GLP-1 only stimulates insulin secretion under hyperglycemic conditions. Intravenous infusions of GLP-1 are able to normalize plasma glucose in patients with type 2 diabetes. Furthermore, hepatic glucose production is lowered by GLP-1 due to the inhibition of glucagon secretion (Figure 2) [15]. A 6-week continuous subcutaneous infusion of GLP-1 significantly decreased glucagon levels and improved glycemic control, HbA1c decreased by 1.3% [24]. Furthermore, the patients treated with GLP-1 lost approximately 2 kg in weight (Figure 3) [24, 37].

Table 1. Favorable effects of incretin mimetics in type 2 diabetes therapy

1.	Potential for normalizing blood glucose/HbA1c.
2.	Glucose dependent effect, less hypoglycemia.
3.	Various principles of action (e.g. glucagonostatic effect).
4.	No dose titration – "one size fits all".
5.	Moderate weight loss possible.
6.	No severe side effects, broad therapeutic range. Nausea as side effect is moderate and transient.
7.	Positive effect on islet cell regeneration/neogenesis: retard the progression of type 2 diabetes?

GLP-1 and its limitations in type 2 diabetes therapy

A possible explanation for the short-lived effectiveness of single subcutaneous injections of GLP-1 was indicated when it was shown that GLP-1 (and the other incretin, GIP) was metabolized by plasma *in vitro* and that the enzyme dipeptidyl peptidase-IV (DPP-IV) was capable of mediating this degradation [38, 39]. Only 20% of the GLP-1 administered during a continuous intravenous infusion is estimated to reach circulation intact as the active form [24, 39]. DPP-IV cleaves peptides with a penultimate amino acid residue alanine or proline leaving biologically inactive split products [38].

Generally, either DPP-IV-resistant peptides that bind to the GLP-1 receptor and show GLP-1-like biological effects or substances inhibiting the DPP-IV could be used to utilize the therapeutic potential of GLP-1 [40].

For this reason, long acting GLP-1 analogs (also referred to as "incretin mimetics") that are resistant to degradation by DPP-IV, are currently being evaluated for clinical use or already being introduced into clinical practice [40-43].

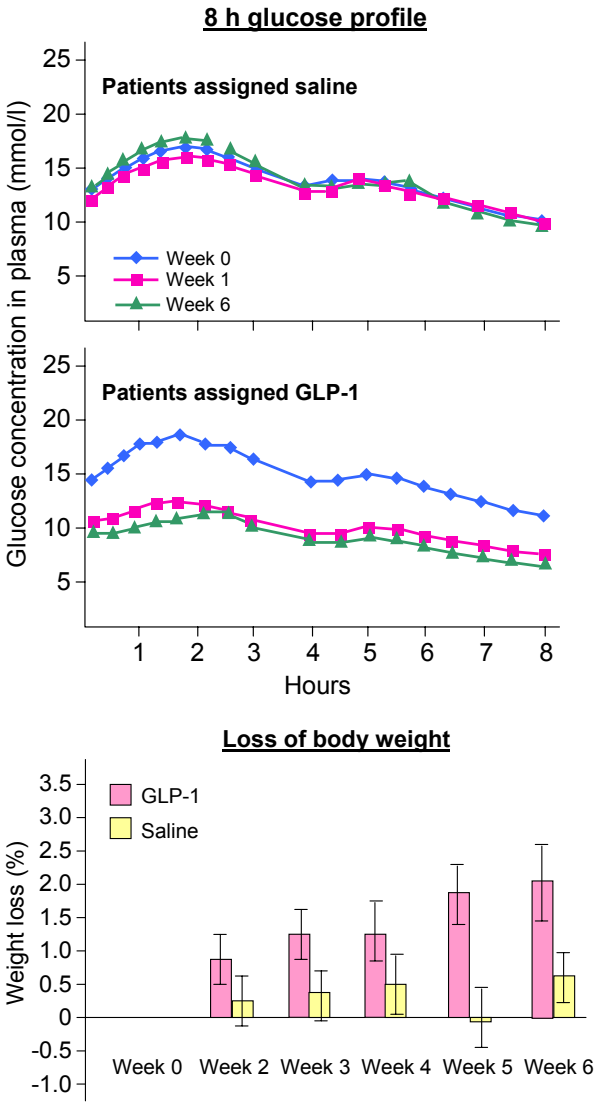

Figure 3. Blood glucose and body weight at baseline, after 1 week and after 6 weeks treatment with either a continuous subcutaneous infusion of GLP-1 or placebo. The graphs show blood glucose profiles over a day during week 0, 1 and 6, the bars represent the loss in body weight in percentages (modified according to [24]).

Advantages of "incretin mimetics" and synthetic GLP-1 analogs as new therapeutic agents

Currently, the first incretin mimetic already available in the U.S.A. for the therapy of type 2 diabetic patients not optimally controlled with oral agents is exenatide (Byetta®, Eli Lilly & Amylin Pharamaceuticals). It is the synthetic form of a naturally occurring peptide called exendin-4 that was originally found in the sali-

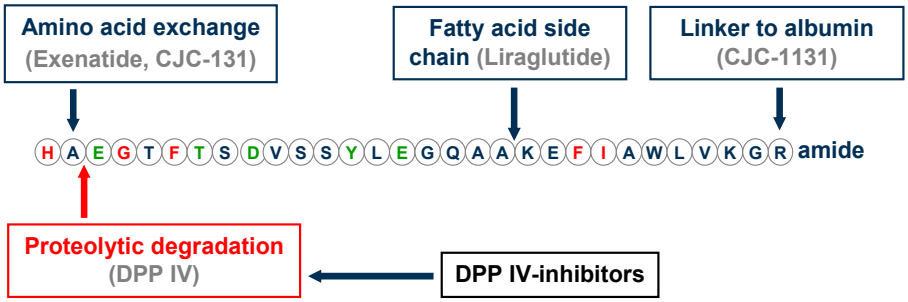

Figure 4. Native GLP-1 and possible molecular changes to create long-acting incretin mimetics. The amino acid sequence of native GLP-1 is shown in the one-letter code. Also, the N-terminal cleavage site of DPP-IV is depicted by an arrow. The schematic molecular changes of the currently available incretin mimetics are shown.

vary gland of the Gila monster (*Heloderma suspectum*). Exenatide has a high amino acid sequence identity to GLP-1 and is not degraded by DPP-IV (Figure 4). It binds to the GLP-1 receptor *in vitro* with a higher affinity than GLP-1 and shows similar gluco-regulatory effects as native GLP-1 [44]. In clinical studies, a twice-daily subcutaneous administration of exenatide resulted in a significant improvement of glycemic control without weight gain and an improvement in β-cell function without causing hypoglycemia in monotherapy (Figure 5) [45, 46]. Hypoglycemia occurred only in patients receiving exenatide and unadjusted doses of sulfonylureas [31, 46-49].

The synthetic GLP-1 analog liraglutide (NN2211) (Novo Nordisk pharmaceuticals) is also DPP-IV resistant and possesses a biologically longer half-life than native GLP-1 due to the addition of a fatty acid side chain to the peptide molecule (Figure 4). The mechanism of protraction is a combination of albumin binding and self-association, resulting in slow absorption from subcutis, stability against dipeptidyl-peptidase IV, and a long plasma half-life. Due to its prolonged action ($t_{0.5}$ = 13 h) it is suitable for once-daily injection. Liraglutide also improves plasma glucose and HbA1c [37, 50]. A recent study reported only one hypoglycemic event in 135 patients (0.074%) receiving liraglutide compared to four of 26 patients (15.4%) receiving sulfonylurea. Another study reported 2.8% of subjects receiving liraglutide experiencing hypoglycemia compared to 5.8% of the group receiving metformin [37, 51]. Recent studies using primary neonatal rat islets showed that native GLP-1 and liraglutide similarly inhibited both cytokine- and free fatty acid-induced apoptosis in a dose-dependent manner, suggesting that liraglutide may be useful for retaining β-cell mass in both type 1 and type 2 diabetic patients [52].

CJC-1131 (under development by ConjuChem) is an analog of GLP-1 with a very long half-life due to its DPP-IV resistance and covalent binding to serum albumin (Figure 4).

All the compounds mentioned lead to an increase of β-cell mass and an improvement of β-cell function in experimental animal models [52-55].

Unlike insulin treatment that requires substantial dose adjustment, there is likely to be a standard therapeutic incretin dose for most patients. Dosing of the incretin mimetics and GLP-1 analogs will probably be uncomplicated because the probability of hypoglycemia is low. Nausea (not more than experienced with metformin therapy) [40, 41, 47] may be observed during the beginning of treatment, but can be controlled with mild antiemetic drugs and usually ceases within a few days. The only disadvantage is that all compounds must be administered subcutaneously, as non-peptidergic incretin mimetics for oral use are not yet available [40].

All GLP-1 analogs and incretin mimetics mentioned in this review have been safe and well tolerated in clinical trials [40, 41]. Nausea occurred in some patients at the beginning of therapy, but it was usually mild and did not lead to a discontinuation of treatment [47]. Hypoglycemia was less common compared to patients receiving oral anti-glycemic medication in these trials [31, 37, 47].

DPP-IV inhibitors

The therapeutic principle of GLP-1 can also be implemented by inhibiting GLP-1 degradation. Support for this approach to therapy also comes from the observations that glucose tolerance is improved in animals in which the enzyme has been genetically deleted [56] and in animals treated with DPP-IV inhibitors [57]. Various substances with DPP-IV inhibition properties that have a good bioavailability after oral ingestion are currently being tested in pre-clinical and clinical trials. One compound already in phase III clinical trials is LAF237, vildagliptin® (Novartis Pharma). In clinical studies, vildagliptin lowered HbA1c in type 2 diabetic patients not treated sufficiently with metformin [58].

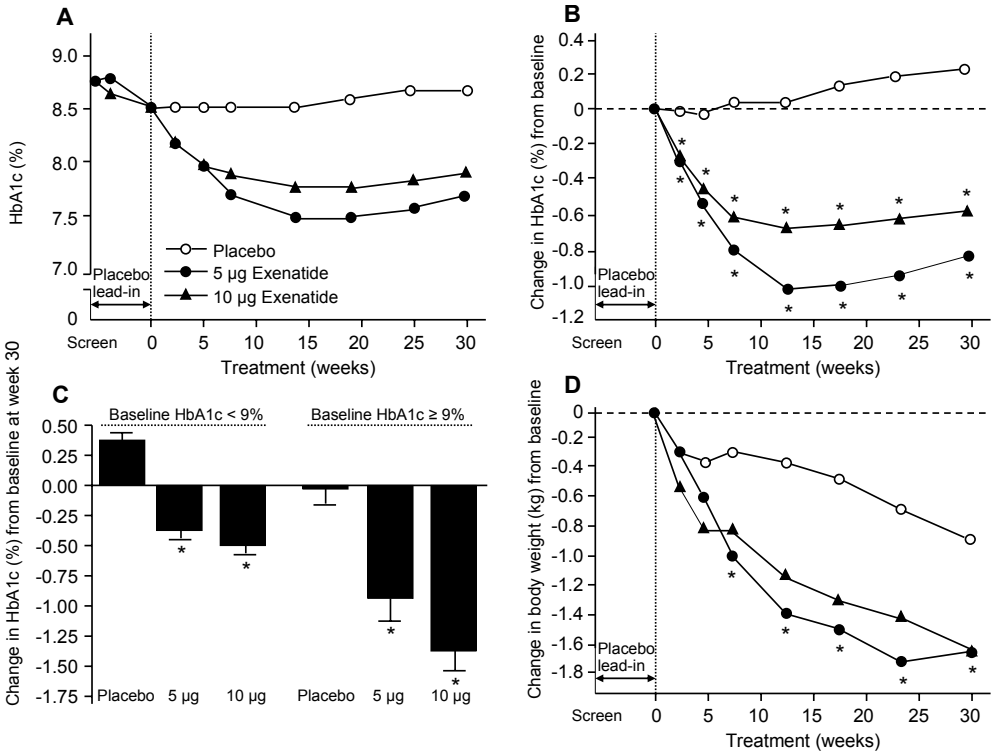

Figure 5. Glycemic control in subjects with type 2 diabetes treated with metformin and a sulfonylurea plus exenatide or placebo (ITT population). A: HbA1c values over the course of the study (raw data). **B**: Change in HbA1c over 30 weeks. * Adjusted $p < 0.0001$ compared with placebo. Week 30 changes in HbA1c values from baseline were -0.77 ± 0.08% (10-µg arm; adjusted $p < 0.0001$ vs. placebo), -0.55 ± 0.07% (5-µg arm; adjusted $p < 0.0001$ vs. placebo), and 0.23 ± 0.07% (placebo arm). **C**: Week 30 change in HbA1c stratified by baseline HbA1c. For subjects with baseline HbA1c < 9%, baseline HbA1c values were 7.92 ± 0.04% (n = 169), 7.91 ± 0.04% (n = 172), and 7.94 ± 0.04% (n = 172) for the 10-µg exenatide, 5-µg exenatide, and placebo arms, respectively. The corresponding values for subjects with baseline HbA1c ≥ 9% were 9.86 ± 0.07% (n = 72), 9.75 ± 0.07% (n = 73), and 9.75 ± 0.07% (n = 75). **D**: Effects of exenatide on body weight. Subjects in the 10-µg exenatide b.i.d. treatment arm received 5 µg exenatide b.i.d. during weeks 0-4. Subjects in all treatment arms were maintained on metformin-sulfonylurea therapy. * $p < 0.001$ compared with placebo treatment. Data are means ± SE (reproduced from [49]).

In contrast to the "incretin mimetics", the weight loss observed with therapy using these substances is not observed with the treatment of DPP-IV inhibitors that seem to be weight neutral. On the other hand, therapy with DPP-IV inhibitors does cause nausea as a side effect, which is observed in therapy with "incretin mimetics". The application of DPP-IV inhibitors retards endogenous GLP-1 degradation, but there is still some uncertainty as to whether all effects of DPP-IV inhibitors are mediated by the prolongation of the biological half-life of the peptide [59-61]. One puzzling finding might support this: in patients with type 2 diabetes, concentrations of active GLP-1 after meal ingestion are doubled by DPP-IV inhibition (compared with placebo), and glucose control improves [58]. In contrast, when similar increases in GLP-1 levels are produced by exogenous infusion, these have little or no effect on insulin secretion or glucose levels [11]. This suggests that mediators other than GLP-1 may contribute to the therapeutic effect of DPP-IV inhibition. For instance, DPP-IV inhibition also blocks the inactivation of the other major incretin hormone, gastric inhibittory peptide (GIP) [59]. Furthermore, various neuropeptides may contribute to the actions of DPP-IV inhibitors in diabetes. These are biologically active peptides that are localized to islet nerve terminals and function as neurotransmitters; some may be substrates for DPP-IV [59]. One neuropeptide of potential importance is pituitary adenylate cyclase-activating peptide (PACAP), which is localized to islet nerves and has several actions relevant to glucose homeostasis [62]. For example, PACAP is a powerful stimulator of insulin secretion and may, like GLP-1, be of importance for islet mass. PACAP may play a leading role in contributing to the prandial, neurally dependent cephalic phase of insulin secretion. Furthermore, it enhances glucose uptake in adipocytes and augments the antilipolytic action of insulin [59, 63]. Since PACAP is also a substrate for DPP-IV, it is reasonable to speculate that this neuropeptide may contribute to the therapeutic benefits of DPP-IV inhibition. However, it is not yet known whether neuropeptides such as PACAP are substrates of DPP-IV in humans under physiological conditions, and this remains a weakness in this line of argument [59].

Because DPP-IV is involved in the degradation of many peptide hormones, the action of DPP-IV is less specific than "incretin mimetics". Along with this, the long-term immunological effects of DPP-IV inhibitors in humans are not yet known, since DPP-IV is also expressed on lymphocytes as CD 26 [64, 65].

In summary, the therapeutic principle of GLP-1 using "incretin mimetics" is a new and attractive treatment option with multiple favorable actions for type 2 diabetes (see Table 1) [40, 41].

Perspectives

The therapeutic principle of GLP-1 with the multiple mode of action besides its glucose-normalizing effect adds a new and attractive perspective to diabetes therapy. Since "incretin mimetics" are peptides, they have to be injected. This fact and their potential costs will probably give them a place in clinical practice for patients who have failed on oral therapy and in whom insulin therapy is not an alternative due to weight problems or possible hypoglycemia. Theoretically, GLP-1-like agents may also be useful in slowing the progression of type 2 diabetes or to be used as anti-obesity agents [66], but here life-style intervention and metformin are also effective [67]. DPP-IV inhibitors have the advantage of being oral (and maybe less costly) agents, but their multiple effects are currently not completely elucidated [59]. So far, only data from clinical trials covering a time frame of little more than one year are available. Long term effects of "incretin mimetics" and DPP-IV inhibitors e.g. on β-cell proliferation and on the brain have to be followed in clinical practice.

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