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MiniReview

Glucagon-Like Peptide-1 Formulation – the Present and Future Development in Diabetes Treatment

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Abstract: Type 2 diabetes mellitus is a chronic metabolic disorder that has become the fourth leading cause of death in the developed countries. The disorder is characterized by pancreatic β-cells dysfunction, which causes hyperglycaemia leading to several other complications. Treatment by far, which focuses on insulin administration and glycaemic control, has not been satisfactory. Glucagon-like peptide-1 (GLP1) is an endogenous peptide that stimulates post-prandial insulin secretion. Despite being able to mimic the effect of insulin, GLP1 has not been the target drug in diabetes treatment due to the peptide's metabolic instability. After a decade-long effort to improve the pharmacokinetics of GLP1, a number of GLP1 analogues are currently available on the market. The current Minireview does not discuss these drugs but presents strategies that were undertaken to address the weaknesses of the native GLP1, particularly drug delivery techniques used in developing GLP1 nanoparticles and modified GLP1 molecule. The article highlights how each of the selected preparations has improved the efficacy of GLP1, and more importantly, through an overview of these studies, it will provide an insight into strategies that may be adopted in the future in the development of a more effective oral GLP1 formulation.

The efficacy of a therapeutic agent lies heavily on a number of factors – the systemic and tissue bioavailability of the agent, the specificity of the agent on target receptor and the side effect profiles after drug administration. The needs to improve the efficacy of a therapeutic agent are particularly pertinent for therapeutics used in the management of chronic diseases such as diabetes. The strategies employed so far can generally be classified into drug delivery technology and gene therapy. Drug delivery technology refers to modification of either a dosage form or a drug formulation of an existing drug substrate, and the technology aimed to improve the drug efficacy and thus the side effect profiles and patient compliance [1].

The application of drug delivery technology to diabetes treatment has been reported for more than a decade, and insulin is the most extensively studied peptide in this context. As such, insulin therapy is not reviewed here. The present Minireview focuses on the formulation and molecular engineering of glucagon-like peptide-1 (GLP1) in the treatment of type 2 diabetes mellitus (T2DM). It is worth noting that the effectiveness of GLP1 in lowering blood glucose levels in type 1 diabetes mellitus (T1DM) has been tested by some researchers, and subcutaneous injection of GLP1 as an add-on to insulin therapy improved glycaemic control in T1DM [2]. However, because T1DM and T2DM differed in terms of the causes and the characteristics of the diseases, insulin is still considered the most effective medication in T1DM, and other potential therapeutic agents such as GLP1 acts as an adjunct therapy [3]. The Minireview presents some of the most promising GLP1 formulations that have been developed so far for use in T2DM, and they include both oral and parenteral preparations.

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Glucagon-Like Peptide-1

The gastrointestinal tract releases more than 20 peptide hormones, two of which are incretins. They are GLP1 and glucose-dependent insulinotropic polypeptide (GIP). By definition, incretins are hormones that are released from enteroendocrine cells and they stimulate insulin secretion. GIP is a 42-amino acid peptide secreted by K cells of the small intestine. Despite being an insulinotropic, patients suffering from T2DM are resistant to GIP, which has thus restricted the use of GIP in the treatment of T2DM.

Glucagon-like peptide-1 is a 30-amino acid peptide secreted by L cells of the small intestine in response to food [4]. It is present in two active forms in the blood circulation, namely GLP1 (7–36) and GLP1 (7–37) amide. GLP1 exerts its insulinotropic effect by binding to GLP1 receptor on the pancreatic β-cells. GLP1 receptor is a G protein-coupled receptor, and activation of the GLP1 signalling pathway occurs only during hyperglycaemia, which means that insulin secretion is regulated by GLP1 in a glucose-dependent manner. GLP1 also suppresses the release of glucagon from the pancreas and glucose production in the liver, and uniquely, GLP1 therapy

does not cause hypoglycaemia and weight gain. Moreover, GLP1 promotes proliferation of pancreatic β-cells, prevents apoptosis of β-cells and delays gastric emptying. The beneficial effects demonstrated by GLP1 show that GLP1 is a promising therapeutic target for T2DM.

However, endogenous GLP1, once secreted, is rapidly cleaved by enzyme dipeptidyl peptidase-IV (DPPIV). Even if GLP1 escapes first-pass metabolism in the intestine, it is exposed to enzymatic degradation in the liver, and later in the systemic circulation, leaving the peptide a mere 2–3 min. half-life [5,6]. The metabolic instability of GLP1 can be overcome by employing drug delivery techniques, as described below.

GLP1 Nanoparticles Formulation

Nanodelivery systems have a number of advantages over microparticles [7]. Briefly, nanoparticles are more suitable for intravenous delivery than microparticles. More importantly, they have relatively higher cellular and intracellular uptake compared to the latter, and because of this, drug delivery through nanoparticles suggests better systemic and tissue bioavailability. The nanosize allows for selective drug accumulation at target sites, and this property will be most beneficial when nanoparticles are conjugated with a tissue- or cell-specific ligand [8]. Because of the versatility of nanoparticles and the potential that nanoparticles have in meeting the criteria of an ideal therapeutic agent, this Minireview focuses on GLP1 nanoparticles that have been developed so far.

Qu *et al.* [9] may be the first to report the preparation of GLP1 nanoparticles. The silica-based pH-sensitive nanomatrix system for oral delivery of GLP1, which is composed of fine silica particles and Eudragit®, was prepared using the rotary evaporation method. The nanomatrix system prolonged the half-life of native GLP1 by three times, and in comparison with GLP1 that was administered intraperitoneally, the formulation increased GLP1 bioavailability by 36% and achieved a 77% hypoglycaemic efficacy. From the everted gut sac study, the transport of GLP1 in the nanomatrix system across the intestinal mucosal layer was five times higher than that of the native GLP1, and this was likely to be contributed by two factors – Eudragit®, which increased the stability of GLP1 against the action of digestive enzymes, and the release profile of GLP1. The nanomatrix system demonstrated a rapid release of GLP1 at pH 7.4, and most of the GLP1 was released in the first hour. A burst release indicates that more GLP1 is available in the intestinal lumen for absorption, although this may also mean that more frequent administration is required compared to a formulation with sustained release property.

Long before the study by Qu *et al.* [9], there was already a study that used biodegradable polymers as a carrier for GLP1 [10]. The formulation was one of the pioneer-controlled release systems developed for GLP1. Researchers prepared a triblock copolymer of poly[(DL-lactic acid-co-glycolic acid)-b-ethylene glycol-b-(DL-lactic acid-co-glycolic acid)] (ReGel) and used that as a depot for the delivery of GLP1. The aqueous solution of the copolymer undergoes a temperature-dependent reversible solution–gel transition. At room temperature, the ReGel and GLP1 mixture forms a solution, which enables it to be injected subcutaneously. Inside the body, that is when temperature increases to 37°C, the copolymer forms a gel that holds the GLP1, and the gel gradually releases the peptide. In this formulation, 60% of GLP1 was released on day 1, and by day 3, 80% of the initial loading was released. Despite the big burst, the system enables a much slower GLP1 release compared to 80% in 12 hr from the silica-based nanoparticles [9]. This much improved formulation is encouraging and suggested that it is only a matter of time that oral GLP1 with a longer blood residence time might be successfully developed.

Liposome is another widely used material in nanoparticle formulation of various drugs, and it has been tested on GLP1. Hanato *et al.* [11] incorporated GLP1 into liposomes by rehydration of the lyophilized empty liposomes with an aqueous GLP1 solution, and anionic liposomes gave the highest encapsulation efficiency of GLP1 compared to non-ionic and cationic liposomes. Intravenous administration of the liposomal GLP1 to rats has resulted in a 1.7 times increase in insulin secretion compared to GLP1 solution. The hypoglycaemic and insulinotropic actions of liposomal GLP1 appeared to be attributed to the improved pharmacokinetics profile of GLP1. Nonetheless, as with the triblock copolymer, the liposomal GLP1 was given by injection, and parenteral route of administration is known to result in poor patient compliance.

The preparation of an oral GLP1 formulation needs to protect GLP1 from enzymatic degradation in the gastrointestinal tract and allows GLP1 to penetrate the mucous layer of the intestine [12]. The latter can be achieved by modifying the surface of the particles using mucoadhesive biomaterials, such as chitosan [13]. When compared to the non-coated nanoparticles, poly(DL-lactic acid-co-glycolic acid) (PLGA) and porous silicon nanoparticles coated with chitosan showed a slower release of GLP1 in the simulated intestinal fluid. The total amount of GLP1 that was released from the chitosan-coated PLGA and silicon nanoparticles after 6 hr was 21% and 35%, respectively. However, since the release tests were carried out for 6 hr only, the release profiles thereafter, as well as the time taken for a complete release of GLP1 from the nanoparticles, were unknown. Chitosan allowed the nanoparticles to interact with the mucous and the membrane of Caco2:HT29-MTX coculture cells, while no such interaction was seen from non-coated nanoparticles. The same study has also shown that chitosan-coated nanoparticles permeated across the cellular monolayer. However, because the permeability study was not done on non-coated nanoparticles, the efficiency of chitosan in this aspect was unclear. Moreover, since evaluation of the mucosal translocation was performed *in vitro*, the above data may not be translated to *in vivo* situation.

Recently, the same group of researchers attempted to address the stability of chitosan-coated PLGA and porous silicon nanoparticles *in vivo* by preparing a GLP1 nanoparticulate system that may withstand the environment in the gastrointestinal tract. By using the aerosol flow reactor technology [14] and microfluidics technique [15], the GLP1-loaded, chitosan-coated nanoparticles were encapsulated into a pH-responsive polymeric nanomatrix, which was also loaded with

a DPPIV inhibitor, forming nano-in-nano systems. As a result, GLP1 released from the nanomatrix system has a much higher permeation across the intestinal cells compared to GLP1 released from the chitosan-coated nanoparticles. The nanomatrix system preserved the properties of chitosan and the DPPIV inhibitor, and allowed the latter to be transported across the cell monolayers. On the other hand, the release of GLP1 was sustained in the PLGA-based system, while 40% of GLP1 was released from the porous silicon nanoparticles during the first 30 min. The burst release from porous silicon nanoparticles increased the amount of GLP1 available in the intestine for absorption and that corresponded with the amount of GLP1 that crossed the cell monolayers [13]. Nevertheless, if we compare these two preparations with the ones without DPPIV inhibitor, the PLGA and the porous silicon nano-in-nano systems increased the permeability of GLP1 to a different extent, that is by five times and 1.5 times, respectively. This suggested that the addition of DPPIV inhibitor into the polymeric nanomatrix improved the absorption of GLP1 from the PLGA nanosystems more than it did for the silicon nanosystems. Moreover, GLP1 may be degrading faster than the action of DPPIV inhibitor since inclusion of the inhibitor only marginally increased the absorption of GLP1 loaded in the silicon nanosystems. Taken together, given the fact that the silicon-DPPIV inhibitor nanosystems gave higher GLP1 permeability, but demonstrated burst release property, which one of these two nano-in-nano systems would be more beneficial for the long-term diabetes treatment is something remains to be considered. As studies were carried out in an *in vitro* cell-based intestinal epithelium model also prompted a few questions. Would the nanomatrix systems function in the same manner *in vivo*? Does the amount of GLP1 and DPPIV inhibitor that crossed the intestine equate the amount in the blood circulation? How efficient is DPPIV inhibitor in suppressing GLP1 degradation in the circulation?

The Modified GLP1

In the triblock copolymer (ReGel) [10], zinc complexed to GLP1 has prevented initial burst of release of GLP1 from the copolymer. The formulation enabled GLP1 to be released linearly and at a constant rate over 2 weeks. Zinc has protected GLP1 from rapid degradation, extended the blood circulatory time of GLP1 and maintained it at a higher level than that of the control group. Zinc-complexed GLP1 retained GLP1 bioactivity as it stimulated insulin secretion and prevented glucose elevation in diabetic rats [10]. The concern of this formulation was obviously the use of zinc. The elimination profile and potential toxic effects of zinc that may come along administration of GLP1-zinc complex need to be addressed, especially in the case of repeat dosing.

Polyethylene glycol (PEG) is one of the most widely used conjugating agents of particles and biomolecules. PEGylation of proteins and peptides is known to improve the pharmacokinetics properties of polypeptides [16]. PEG conjugation to GLP1 was first reported by Lee *et al.* [17]. The PEGylated GLP1 was resistant to DPPIV *in vitro*, and when administered intravenously and subcutaneously, GLP1 showed longer plasma half-life, lower clearance rate and an increased mean plasma residence time. GLP1 peptide sequence, which has Lys^{34} conjugated with PEG, was as potent as native GLP1 in stimulating insulin secretion in the islets and was more effective than GLP1 in lowering glucose levels after intraperitoneal injection [18]. The hypoglycaemic effect of Lys^{34}-PEGylated GLP1 was reproducible when the modified GLP1 was administered intranasally [19].

Biotin has been used in the preparation of oral GLP1 [20]. Biotin or vitamin H is absorbed from the intestine via the sodium-dependent multivitamin transporter, and the transporter was reported to facilitate intestinal peptide uptake [21]. Biotinylated GLP1 was prepared by conjugating biotin specifically to Lys^{26} and Lys^{34} sites of the GLP1. The biotinylated GLP1 permeated across the Caco-2 cell monolayer six times more efficient than GLP1, was more stable than GLP1 in the rat intestinal fluid and homogenate and showed a 20% higher oral hypoglycaemic efficacy than GLP1 [20]. PEGylation of the biotinylated GLP1 (Lys^{26}-biotin-Lys^{34}-(biotin-PEG)-GLP1) further increased the stability of GLP1 in the biological fluid. A surge in plasma GLP1 levels was observed within the first hour after oral administration of the biotin-PEGylated GLP1 [22]. However, in the same study, although biotinylated GLP1 has again shown to have better stability than native GLP1 in the intestinal fluid, plasma GLP1 remained at basal level, which implied negligible intestinal absorption. Therefore, the hypoglycaemic effect of the biotinylated GLP1 reported earlier [20] may not be contributed by or at least not entirely by the formulation. Further investigation is necessary to explain the mechanism of action of the biotinylated GLP1.

PEGylation at sites other than Lys and His [18], together with modification of the peptide sequence, has been attempted [23]. Double substitutions of Ala^{8} and Ala^{30} with Gly and Cys, respectively, followed by PEG conjugation of Cys^{30}, allowed the modified GLP1 to remain stable against DPPIV after 4 hr of incubation with the enzyme, and following intraperitoneal injection moderately reduced glucose levels [23]. Other approaches to maintain the stability of GLP1 include conjugation of GLP1 with albumin [24] and amphipathic peptide [25], and formation of a GLP1 homodimeric structure [26]. These studies are summarized in table 1. Table 1 also presents other findings described earlier so that it allows us to compare the bioavailability and the potency of GLP1 from the formulation reported so far, and the advantages and disadvantages of each formulation, and gives an insight into strategy that may further improve the efficacy of GLP1.

Challenges Faced and Future Investigation

Despite the fact that oral formulation is preferred over injectable formulation, specifically in terms of ensuring patient compliance, the development of GLP1 formulation for parenteral administration appeared to predominate, as reviewed above. This may be due to the perceived greater success rate in increasing GLP1 bioavailability from an injection than to an

Table 1.
Biological properties of glucagon-like peptide-1 (GLP1) nanoparticles and modified GLP1 from selected studies.

Preparation	GLP1 concentration	Route of administration	Subject	Half-life of GLP1 (*in vitro*)	Peak plasma or serum GLP1 level	Insulinotropic effect	Hypoglycaemic effect	Other significant findings
GLP1-loaded silica-based pH-sensitive nanomatrix system [9]	1 mg GLP1/kg body-weight	Oral	Sprague Dawley rat	~3 times longer than native GLP1 in the rat intestinal fluid	2.21 ng/mL	–	77% efficiency *versus* i.p. native GLP1	A five times increased permeability across rat's intestinal mucosa; plasma GLP1 returned to basal level after 4 hr
Zinc-complexed GLP1-loaded PLGA-polyethylene glycol (PEG)-PLGA copolymers [10]	10 mg GLP1/rat	Subcutaneous (single injection)	Zucker diabetic fatty rat	–	200 ng/L	Peak at 4.5 μg/mL on days 1 and 2	Glucose level reduced to ~200 mg/dL in the first 2 days after injection	Induced insulin secretion and lowered glucose levels for 15 days, corresponded with elevated plasma GLP1 levels
Liposomal formulation of GLP1 [11]	100 nmol/kg	Intravenous	Wistar rats	–	4.4 μg/mL at 15 min.	1.7 times higher blood insulin (pg/mL) than GLP1	Reduced glucose level by 30% *versus* control	–
GLP1-loaded chitosan-coated porous silicon nanoparticles [13]	–	*In vitro*	–	–	–	–	–	Nanoparticles interacted with intestinal mucous and cells; high permeation across Caco-2:HT29-MTX: Raji B monolayers
GLP1-loaded chitosan-coated nanoparticles in dipeptidyl peptidase-IV (DPPIV) inhibitor-containing nanomatrix system [14,15]	–	*In vitro*	–	–	–	–	–	Increased permeability of GLP1 *versus* nanoparticulate system without DPPIV inhibitor
Lys^{34}-PEGylated GLP1 [18]	9 nmol GLP1/kg	Intraperitoneal (single injection)	C57BL/6 db/db mice	40 times longer than native GLP1 in plasma	–	Equally effective as native GLP1 in stimulating insulin secretion from isolated rat pancreatic islets (*in vitro*)	Reduced basal glucose level by 60% after 1-hr injection	The reduced glucose level was maintained for 5 hr

(continued)

Table 1. (continued)

Preparation	GLP1 concentration	Route of administration	Subject	Half-life of GLP1 (*in vitro*)	Peak plasma or serum GLP1 level	Insulinotropic effect	Hypoglycaemic effect	Other significant findings
$Lys^{26,34}$-biotin-GLP1 [20]	15 nmol GLP1/mouse	Oral	db/db mice	8.5 and 3.5 times longer than native GLP1 in rat intestinal fluid and homogenate	–	Comparable activity with native GLP1 in the rat islets (*in vitro*)	Nine times higher efficacy than native GLP1; effect lasted 180 min.	5.6 times higher Caco-2 cell monolayer permeability than native GLP1
Lys^{26}-biotin-Lys^{34}-(biotin-PEG)-GLP1 [22]	15 nmol GLP1/mouse; 20 nmol/rat in pharmacokinetics study	Oral	db/db mice; Sprague Dawley rat	24 times (or ~16 min.) and 9.9 times (exceed 60 min.) longer than native GLP1 in intestinal fluid and DPPIV solution, respectively	7.72 ng/mL	Comparable activity with native GLP1 in the isolated rat islets	28% efficiency *versus* native GLP1; effect lasted 180 min.	Plasma half-life was seven times higher than i.v. native GLP1; plasma GLP1 returned to basal level after 2 hr
Lys^{34}-PEGylated GLP1 [19]	100 nmol GLP1/kg	Intranasal	C57BL/6 db/db mice	Increased 2.4 to 11 times in the rabbit nasal mucosa homogenates	–	–	Hypoglycaemic effect up to 2 hr	–
PEGylated-Gly^{8}-Cys^{30}-GLP1 [23]	25 nmol GLP1/kg	Intraperitoneal	Kunming mice	Remained intact after 4-hr incubation with DPPIV	–	–	31% efficiency compared to native GLP1 at 30 min.	Glucose-lowering effect lasted 1 hr
Albumin-conjugated GLP1 [24]	100 nmol GLP1/kg	Subcutaneous	db/db mice	–	–	Did not induce insulin secretion	Glucose level reduced to ~5 mmol/L in the first 2 days after injection and remained reduced for 10–12 hr	Glucose level remained low 1 week after discontinuation of the 4 weeks treatment regimen comprised of twice daily intraperitoneal injection at 25 μg GLP1
GLP1/peptide 1 complex [25]	100 μg GLP1	Subcutaneous	Sprague Dawley rat	90 hr *versus* 2.5 min. for native GLP1 in human serum	~30 μg after a single subcutaneous injection of the complex containing 750 μg GLP1	957 pmol/L *versus* 854 pmol/L from native GLP1	Reduced elevated glucose level to ~7 mmol/L within 5 min. and the level was maintained for 30 min.	–

(continued)

Table 1. (continued)

Preparation	GLP1 concentration	Route of administration	Subject	Half-life of GLP1 (*in vitro*)	Peak plasma or serum GLP1 level	Insulinotropic effect	Hypoglycaemic effect	Other significant findings
Disulphide bridged GLP1 homodimeric analogue [26]	100 μg GLP1	Subcutaneous	Sprague Dawley rat	Amount still detected in human serum after 24 hr and was 5.4 times more than that of native GLP1	55 μg after a single subcutaneous injection of 100 μg GLP1	Secreted 1.8 times more insulin than native GLP1 after subcutaneous injection of 200 μg GLP1	Reduced glucose level to ~6 mmol/L within 5 min. and the level was maintained for 30 min.	–

oral intake. In the gastrointestinal tract, GLP1 should be resistant to proteolytic degradation and is able to cross the intestinal barrier. For the modified GLP1, the bioactivity of GLP1 needs to be preserved as well.

The oral GLP1 formulation reported so far was able to prolong the half-life of GLP1 from 3 to 24 times [9,20,22], and among these studies, the modified GLP1 has longer half-life than native GLP1 loaded in the silica-based pH-sensitive nanomatrix system. While the biotin-PEGylated GLP1 has a half-life of 16 min. [22], the biotinylated GLP1 was completely degraded in 16 min. [20]. *In vivo*, these formulations demonstrated significant hypoglycaemic effect that lasted for about 3 hr. The duration of action was considered short compared with the injectable zinc-complexed GLP1 loaded in ReGel [10], but was longer than some other injectable modified GLP1s [19,23,25,26]. Despite the initial burst of GLP1, the silica-based nanomatrix system elevated plasma GLP1 level for 4 hr [9], while it was only 2 hr from the biotin-PEGylated GLP1 [22]. Together, data suggested that an injectable formulation may not necessarily give a better pharmacokinetics profile and bioactivity than an oral formulation. In addition, it is possible that not all the injectable GLP1 exhibit their effects after administration [9], because systemic bioavailability may not be equivalent to tissue bioavailability.

Encapsulation of a modified GLP1 or a GLP1 analogue either in nanoparticles or a nanomatrix system may possess the biological properties we hope to achieve. A well-formulated carrier system allows a sustained release of GLP1 from the depot and prolongs the blood residence time of GLP1. The property of the system may be further enhanced by complexing GLP1 with a stabilizing molecule, which prevents GLP1 from being rapidly degraded once it is released from the depot, thereby also improves the bioavailability of GLP1. However, the possible drawback could be that the cost of production increases in parallel with the complexity of the formulation process.

To date, the progress made in developing an oral GLP1 has been encouraging. Nonetheless, while setting the goal to develop an 'ideal' oral GLP1, we must not forget that prolonged elevation in plasma GLP1 is not required. The glucose-lowering effect of GLP1 is concentration dependent. This means that as the glucose concentration decreases, GLP1 will no longer function as a hypoglycaemic agent [9]. It is important that the 'unused' GLP1 is eliminated from the body to minimize potential adverse effects of GLP1. So far, research to examine the cause of adverse effects is lacking and this should be emphasized in the future especially given the fact that the adverse effects can potentially compromise the beneficial effects of GLP1 treatment. For example, acute pancreatitis has been reported during the treatment of GLP1-based drugs [27], and patients with diabetes have a two to three times increased risk of acute pancreatitis compared to individuals who do not have diabetes mellitus [28]. There were cases of increased heart rate in patients receiving GLP1 analogues [29,30], although the incidence may not lead to severe cardiovascular events. Nevertheless, continuous activation of GLP1

receptor did result in a greater increase in heart rate than intermittent stimulation of the receptor [31]. In short, there should be a balance between achieving the optimal therapeutic effect of GLP1 from a sustained release oral formulation and the risk of side effects.

Conclusion

GLP1 displays the ability to improve glycaemic control in diabetes mellitus and uniquely does not cause hypoglycaemia. However, the therapeutic use of GLP1 is limited due to it being highly unstable in the physiological system. Drug delivery system is a potential solution to overcome this issue, and it has not been widely explored [9]. It is worth mentioning that oral delivery of other macromolecules is facing the same challenge as GLP1 [9]. Given that GLP1 receptor agonists have been found to be more superior to other established therapies in helping patients to achieve their target glucose levels [32] and that oral administration of GLP1 has shown promising therapeutics outcome, further improvement of the oral GLP1 formulation is worth pursuing. Of note, until more extensive animal study is done on the reported formulation, and before human data become available, it is pre-mature for us to judge which one of these formulations is better than the rest.

Conflict of Interest

There was no conflict of interest.

References

1 Sadrzadeh N, Glembourtt MJ, Stevenson CL. Peptide drug delivery strategies for the treatment of diabetes. J Pharm Sci 2007;**96**:1925–54.

2 Behme MT, Dupré J, McDonald TJ. Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. BMC Endocr Disord 2003;**3**:3.

3 Unger J. Rationale use of GLP-1 receptor agonists in patients with type 1 diabetes. Curr Diab Rep 2013;**13**:663–8.

4 Herrmann C, Göke R, Richter G, Fehmann HC, Arnold R, Göke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion 1995;**56**:117–26.

5 Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-$1_{7\text{-}36}$ amide, peptide histidine methionine and is responsible for their degradation in human serum. Eur J Biochem 1993;**214**:829–35.

6 Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 1995;**136**:3585–96.

7 Singh R, Lillard JW. Nanoparticle-based targeted drug delivery. Exp Mol Pathol 2009;**86**:215–23.

8 Lamprecht A, Ubrich N, Yamamoto H, Schäfer U, Takeuchi H, Maincent P *et al.* Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. J Pharmacol Exp Ther 2001;**299**:775–81.

9 Qu W, Li Y, Hovgaard L, Li S, Dai W, Wang J *et al.* A silica-based pH-sensitive nanomatrix system improves the oral absorption and efficacy of incretin hormone glucagon-like peptide-1. Int J Nanomedicine 2012;**7**:4983–94.

10 Choi S, Baudys M, Kim SW. Control of blood glucose by novel GLP-1 delivery using biodegradable triblock copolymer of PLGA-PEG-PLGA in type 2 diabetic rats. Pharm Res 2004;**21**:827–31.

11 Hanato J, Kuriyama K, Mizumoto T, Debari K, Hatanaka J, Onoue S *et al.* Liposomal formulations of glucagon-like peptide-1: improved bioavailability and anti-diabetic effect. Int J Pharm 2009;**382**:111–6.

12 Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv Drug Deliv Rev 2012;**64**:557–70.

13 Araújo F, Shrestha N, Shahbazi M, Fonte P, Mäkilä EM, Salonen JJ *et al.* The impact of nanoparticles on the mucosal translocation and transport of GLP-1 across the intestinal epithelium. Biomaterials 2014;**35**:9199–207.

14 Shrestha N, Shahbazi M, Araújo F, Mäkilä EM, Raula J, Kauppinen EI *et al.* Multistage pH-responsive mucoadhesive nanocarriers prepared by aerosol flow reactor technology: a controlled dual protein-drug delivery system. Biomaterials 2015;**68**:9–20.

15 Araújo F, Shrestha N, Shahbazi M, Liu D, Herranz-Blanco B, Mäkilä EM *et al.* Microfluidic assembly of a multifunctional tailorable composite system designed for site specific combined oral delivery of peptide drugs. ACS Nano 2015;**9**:8291–302.

16 Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. Adv Drug Deliv Rev 2002;**54**:459–76.

17 Lee SH, Lee S, Youn YS, Na DH, Chae SY, Byun Y *et al.* Synthesis, characterization, and pharmacokinetic studies of PEGylated glucagon-like peptide-1. Bioconjug Chem 2005;**16**:377–82.

18 Lee S, Youn YS, Lee SH, Byun Y, Lee KC. PEGylated glucagon-like peptide-1 displays preserved effects on insulin release in isolated pancreatic islets and improved biological activity in db/db mice. Diabetologia 2006;**49**:1608–11.

19 Youn YS, Jeon JE, Chae SY, Lee S, Lee KC. PEGylation improves the hypoglycaemic efficacy of intranasally administered glucagon-like peptide-1 in type 2 diabetes db/db mice. Diabetes Obes Metab 2008;**10**:343–6.

20 Youn YS, Chae SY, Lee S, Kwon MJ, Shin HJ, Lee KC. Improved peroral delivery of glucagon-like peptide-1 by site-specific biotin modification: design, preparation, and biological evaluation. Eur J Pharm Biopharm 2008;**68**:667–75.

21 Russell-Jones GJ, Westwood SW, Habberfield AD. The use of vitamin B12 transport system as a carrier for the oral delivery of peptides, proteins and nanoparticles. Proc Int Symp Control Release Bioact Mater 1996;**23**:49–50.

22 Chae SY, Jin CH, Shin HJ, Youn YS, Lee S, Lee KC. Preparation, characterization, and application of biotinylated and biotin-PEGylated glucagon-like peptide-1 analogues for enhanced oral delivery. Bioconjug Chem 2008;**19**:334–41.

23 Chi Y, Zhang H, Huang W, Zhou J, Zhou Y, Qian H *et al.* Microwave-assisted solid phase synthesis, PEGylation, and biological activity studies of glucagon-like peptide-1(7-36) amide. Bioorg Med Chem 2008;**16**:7607–14.

24 Kim JG, Baggio LL, Bridon DP, Castaigne JP, Robitaille MF, Jetté L *et al.* Development and characterization of a glucagon-like peptide 1-albumin conjugate. Diabetes 2003;**52**:751–9.

25 Zheng X, Li Y, Li X, Tang L, Xu W, Gong M. Peptide complex containing GLP-1 exhibited long-acting properties in the treatment of type 2 diabetes. Diabetes Res Clin Pract 2011;**93**:410–20.

26 Li Y, Li X, Zheng X, Tang L, Xu W, Gong M. Disulfide bond prolongs the half-life of therapeutic peptide-GLP-1. Peptides 2011;**32**:1400–7.

27 Elashoff M, Matveyenko AV, Gier B, Elashoff R, Butler PC. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide 1-based therapies. Gastroenterology 2011;**141**:150–6.

28 Noel RA, Braun DK, Patterson RE, Bloomgren GL. Increased risk of acute pancreatitis and biliary disease observed in patients with

type 2 diabetes: a retrospective cohort study. Diabetes Care 2009;**32**:834–8.

29 Diamant M, van Gaal L, Stranks S, Northrup J, Cao D, Taylor K *et al.* Once weekly exenatide compared with insulin glargine titrated to target in patients with type 2 diabetes (DURATION-3): an open-label randomised trial. Lancet 2010;**375**:2234–43.

30 Pratley RE, Nauck M, Bailey T, Montanya E, Cuddihy R, Filetti S *et al.* Liraglutide versus sitagliptin for patients with type 2 diabetes who did not have adequate glycaemic control with metformin: a 26-week, randomised, parallel-group, open-label trial. Lancet 2010;**375**:1447–56.

31 Meier JJ. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. Nat Rev Endocrinol 2012;**8**:728–42.

32 Zinman B, Schmidt WE, Moses A, Lund N, Gough S. Achieving a clinically relevant composite outcome of an HbA_{1c} of <7% without weight gain or hypoglycaemia in type 2 diabetes: a meta-analysis of the liraglutide clinical trial programme. Diabetes Obes Metab 2012;**14**:77–82.