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Absorption of glucagon-like peptide-1 can be protracted by zinc or protamine

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Abstract

Pharmacokinetics of several different preparations for subcutaneous administration of GLP-1(7-36)amide (GLP-1) were studied. The difference between soluble GLP-1 at pH 4.0 and a suspension of GLP-1 crystals at pH 6.9, and how addition of zinc chloride or protamine sulphate to the GLP-1 suspension affected the absorption kinetics after s.c. administration, were investigated. Four Beagle dogs received 50 μg/kg of GLP-1 s.c. in different vehicle compositions. The vehicle contained 1 mg/ml of GLP-1, 10 mmol/l disodium phosphate, 16 mg/ml glycerol, and in some preparations either zinc chloride or protamine sulphate. The preparations were: (1) pH 4.0, (2) pH 6.9, (3) 1 equiv. (relative to GLP-1) zinc chloride pH 6.9, (4) 0.5 equiv. zinc chloride pH 6.9, (5) 3 equiv. zinc chloride pH 6.9, (6) 0.5 mg/ml protamine sulphate pH 4.0, and (7) 0.5 mg/ml protamine sulphate pH 6.9. Plasma samples were analyzed employing a sandwich enzyme-linked immunosorbant assay. Addition of zinc chloride or protamine sulphate to the GLP-1 suspension decreased the absorption rate. The plasma concentration of GLP-1 was increased to a pharmacological level for at least 15 h when administered s.c. as a zinc or protamine suspension. Furthermore, the peak plasma concentration was flattened out, when the peptide was administered as a suspension containing zinc chloride or protamine sulphate, compared to a solution.

Keywords: Glucagon-like peptide-1; GLP-1(7-36)amide; S.c. preparations; Zinc; Protamine; Pharmacokinetics

1. Introduction

Glucagon-like peptide-1, GLP-1, is a recently discovered gut hormone (Ørskov, 1992). Recently, several studies have shown that exogenous GLP-1 has a potent glucose lowering effect in non insulin dependent diabetes mellitus (NIDDM) patients when infused intravenously to 3–5 times the increase in plasma levels as seen in connection with a meal (Gutniak et al., 1992; Elliott et al., 1993; Nauck et al., 1993). It has also been shown that the plasma concentration profile of GLP-1 desired should show pharmacological concentrations (10–50 pmol/l above basal value) for more than 4 h (Nauck et al., 1993; Deacon et al., 1995)

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to be able to obtain an effect. These results hold promise for GLP-1 as a potential new drug in the management of NIDDM and it is currently considered to be the most promising new concept (Galloway and Chance, 1994; Rachmann and Turner, 1995). However, for GLP-1 to be used as a new drug, a preparation for s.c. administration has to be developed.

When once daily administration is desired, pharmacological concentrations should be achieved for more than 10 h. Additionally, a smoothing of the initial peak plasma concentration must be achieved, to avoid side effects such as delayed gastric emptying and secretion (Wettergren et al., 1993). The plasma profile of GLP-1 after s.c. administration both in humans and dogs as simple solutions showed pharmacological plasma concentrations as a sharp peak for 1–3 h only (Gutniak et al., 1994; Deacon et al., 1995; Pridal et al., 1996). Thus, the absorption rate must be protracted. This is achieved for insulin and glucagon by addition of zinc chloride and/or protamine sulphate to a suspension of crystals (Trading et al., 1969; Pingel et al., 1993).

The present study was undertaken in order to obtain pharmacokinetic data in dogs following subcutaneous dosing of various GLP-1 preparations.

2. Materials and methods

2.1. Animals

Four Beagle dogs, two of each gender, weighing between 14 kg and 19 kg were included in the study. The dogs were fasted from the evening before the studies with free access to water.

2.2. Study drug

GLP-1 (HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRamide) was prepared by solid phase synthesis and purified by preparative reversed phase HPLC as previously described (Adelhorst et al., 1994). The purity, determined by capillary electrophoresis and analytical HPLC was >95%. The molecular weight of 3298 g/mol was confirmed by plasma desorption mass spectrometry.

2.3. Dosing solution for i.v. dosing

First, 0.2 mg/ml GLP-1 was dissolved in 10 mmol/l disodium phosphate containing 16 mg/ml of glycerol and pH was adjusted to 4.0. The solution was subjected to sterile filtration. Then, 125 μl/kg was given to each dog (25 μg/kg).

2.4. Dosing preparations for s.c. dosing

First, 1 mg/ml GLP-1 was dissolved in 10 mmol/l disodium phosphate containing 16 mg/ml of glycerol and pH was adjusted to 4.0. Zinc chloride or protamine sulphate was added to the desired solutions, which were subjected to sterile filtration. The pH of some solutions was adjusted to 6.9 and the preparations were left at room temperature for 3–4 days. Crystal formation was observed in all preparations with pH 6.9, while all preparations with pH 4.0 were stable as solutions. Finally, 50 μl/kg was given to each dog (50 μg/kg). The preparations were: (1) pH 4.0 (GLP-1 solution), (2) pH 6.9 (GLP-1 suspension), (3) 1 equiv. (relative to GLP-1) zinc chloride pH 6.9 (1 equiv. zinc GLP-1 suspension), (4) 0.5 equiv. zinc chloride pH 6.9 (0.5 equiv. zinc GLP-1 suspension), (5) 3 equiv. zinc chloride pH 6.9 (3 equiv. zinc GLP-1 suspension), (6) 0.5 mg/ml protamine sulphate pH 4.0 (protamine GLP-1 solution), and (7) 0.5 mg/ml protamine sulphate pH 6.9 (protamine GLP-1 suspension).

2.5. Dosing and blood sampling

The study was performed on eight separate days 1–3 weeks apart. The dogs were suspended in a harness and venipuncture was performed in a foreleg using a teflon catheter (Venflon 2 18G/1.2 mm OD, Viggo-Spectramed, Sweden, cat. No 1457-1) which was flushed using a solution of heparin (50 i.e./ml prepared in saline). Subcutaneous doses were given in the neck using a syringe, and intravenous doses were administered in a foreleg vein as 2 min infusions using a syringe. Following s.c. administration blood was collected before and 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 15 h after injection. Following i.v. administration blood was collected before and 0, 5, 7.5, 10, 12.5,

15, 30, 45, 60, 90, 120, 150 and 180 min after infusion. Blood (2 ml) was collected in pre-chilled (0°C) polyethylene vials (Minisorp, Nunc, Roskilde, Denmark) each containing 40 μl anticoagulant/stabilizer (0.3 mol/l EDTA, 3.7 mg/ml aprotinin NOVO, pH 7.4). The vials were capped, slowly mixed and placed on ice until centrifugation (4°C, 10 min, 2000 × *g*) which was performed within 30 min of blood collection. Samples were stored at −20°C until analyzed with a sandwich enzyme-linked immunosorbent assay (ELISA).

2.6. Sandwich ELISA procedure

The ELISA procedure has been described in more detail elsewhere (Pridal et al., 1995). The antibody combination used has been shown to result in a cross-reaction of > 10% with GLP-1(7-35) and GLP-1(7-34), of 1–10% with GLP-1(7-33), 0.1–1% with GLP-1(8-36)amide and GLP-1(9-36)amide, and < 0.1% with GLP-1(7-32), GLP-1(7-31), GLP-1(10-36)amide, GLP-1(11-36)amide and glucagon.

2.7. HPLC analyses

HPLC column: Superspher 100 RP − 18 endcapped (E. Merck, Darmstadt, Germany). Eluent: 39% acetonitrile and 0.1% trifluoroacetic in water. Wavelength: 280 nm. Aliquots of 25 μl were injected onto the column. The retention time of GLP-1 was 3–4 min.

2.8. Pharmacokinetic calculations

The plasma concentrations were corrected for the endogenous level, by subtraction of the mean result from the before and 0 min sample. Individual data from i.v. infusions were fitted to two-compartment open models. Individual data from s.c. doses were either fitted to two-compartment open models, or non-compartmental analyses. All calculations were performed by the software Topfit 2.0 (Heinzel et al., 1993).

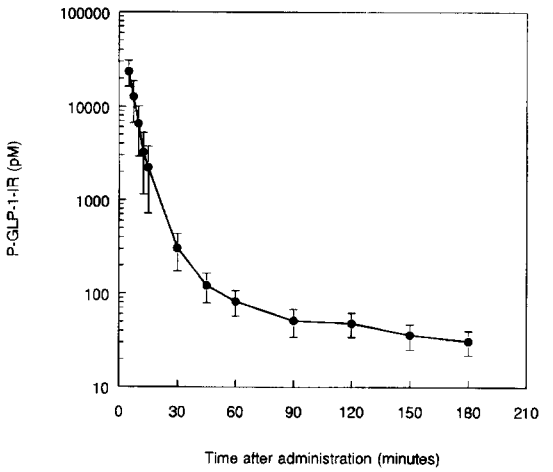

Fig. 1. Plasma GLP-1 immunoreactivity (pmol/l) in Beagle dogs following a single intravenous 2 min infusion of GLP-1 25 μg/kg. $N = 4$. Data are mean ± S.E.M.

2.9. Abbreviations

AUC, the area under the curve; *CL*, the total plasma clearance; C_{max}, the maximum plasma concentration reached; *D*, the dose; *f*, the absolute bioavailability; $t_{1/2\lambda 1}$, the plasma half-life in the first-phase; $t_{1/2}$, the plasma half-life in the final phase; t_{max}, the time to reach C_{max}; V_{ss}, the volume of distribution at steady state.

3. Results

The plasma concentration profile from the i.v. study is shown in Fig. 1. The calculated pharmacokinetic parameters from the i.v. and s.c. studies, respectively, are given in Table 1 and Table 2. All data shown are mean ± S.E.M.

Table 1
Pharmacokinetic parameters in Beagle dogs obtained following a single intravenous 2 min infusion of GLP-1 25 μg/kg

AUC/D	45 ± 9	(pmol/l)·min/pmol
V_{ss}	0.15 ± 0.02	l/kg
CL	25 ± 4.2	ml/(kg·min)
$t_{1/2\lambda 1}$	2.4 ± 0.6	min
$t_{1/2}$	50 ± 4	min

$N = 4$. Data are mean ± S.E.M.

Table 2
Pharmacokinetic parameters in Beagle dogs obtained following single subcutaneous bolus injections of GLP-1 50 μg/kg, as a solution, a suspension, a suspension containing 0.5 equiv. zinc chloride, a suspension containing 1 equiv. zinc chloride, a suspension containing 3 equiv. zinc chloride, a solution containing 0.5 mg/ml of protamine sulphate, or as a suspension containing 0.5 mg/ml of protamine sulphate

	AUC/D ((pmol/l)·min/pmol)	C_{max} (pmol/l)	t_{max} (h)	$t_{1/2(1)}$ (min)	$t_{1/2}$ (h)	f (%)
Solution	20 ± 9	2046 ± 759	0–1	29 ± 6	7.1 ± 0.8	41 ± 10
Suspension	14 ± 5^{NS}	1073 ± 490^{NS}	0–1^{NS}	13 ± 3^{NS}	3.9 ± 0.4^{NS}	30 ± 5^{NS}
1 Equiv. zinc suspension	>4.4 ± 1.2	189 ± 26*	0–1^{NS}			>11 ± 2
		82 ± 23	8–15			
0.5 Equiv. zinc suspension	>4.3 ± 1.3	157 ± 59*	0.5–2^{NS}			>10 ± 1
		88 ± 25	6–8			
3 Equiv. zinc suspension	>2.6 ± 0.6	81 ± 20*	0.5–2^{NS}			>6 ± 1
		47 ± 10	6–15			
Protamine solution	10 ± 4^{NS}	2269 ± 861^{NS}	0–1^{NS}	14 ± 2^{NS}	6.0 ± 1.2^{NS}	24 ± 10^{NS}
Protamine suspension	>5.3 ± 1.3	215 ± 40*	0–1^{NS}			>13 ± 2
		122 ± 32	4–8			

N = 4. Data are mean ± S.E.M.
Significance level compared to the GLP-1 solution are shown in superscript.
^{NS}, non significant.
*P < 0.05.

3.1. The GLP-1 solution and the GLP-1 suspension

The plasma concentration profiles are shown in Fig. 2. The $t_{1/2(1)}$ and $t_{1/2}$, from both the solution and the suspension, were higher than the same parameters from the i.v. study. The absorption rate was independent of whether GLP-1 was administered s.c. in solution or as a suspension. The plasma concentration of GLP-1 was increased above a pharmacological level from 0.5 to 15 h after administering the peptide s.c. No significant difference between the two plasma concentration profiles were detected. Visual inspection of the suspension with a microscope revealed needle-shaped crystals with a length of 3–4 μm and, in addition, amorphous precipitation. Analyses of the supernatant by HPLC revealed that about 90% of GLP-1 was precipitated.

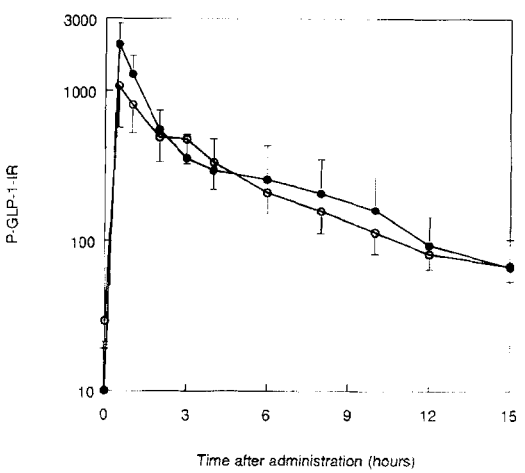

Fig. 2. Plasma GLP-1 immunoreactivity (pmol/l) in Beagle dogs following a single subcutaneous injection of GLP-1 50 μg/kg, as a solution (closed circles) or as a suspension of crystals (open circles). N = 4. Data are mean ± S.E.M.

3.2. Zinc GLP-1 suspensions

The plasma concentration profiles are shown in Fig. 3. The absorption rate was significantly decreased when the suspension contained either 0.5, 1 or 3 equiv. zinc chloride compared to both a GLP-1 solution and a GLP-1 suspension. All the plasma concentration profiles, both the individual and the mean, showed signs of a two phase absorption after injection of the zinc GLP-1 suspensions. $t_{1/2}$ could not be calculated after injection of

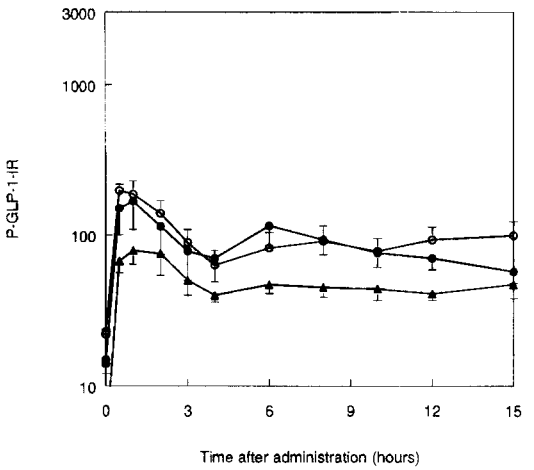

Fig. 3. Plasma GLP-1 immunoreactivity (pmol/l) in Beagle dogs following a single subcutaneous injection of GLP-1 50 μg/kg, as a suspension of crystals containing 0.5 (closed circles), 1 (open circles) and 3 equiv. (closed triangles) zinc chloride, respectively. $N = 4$. Data are mean ± S.E.M.

the zinc GLP-1 suspension because the elimination phase could not be detected within 15 h. The plasma concentrations of GLP-1 were increased to a pharmacological level from 0.5 to 15 h after injecting the peptide s.c. irrespective of zinc chloride concentration. Microscopy of the zinc GLP-1 suspensions revealed needle-shaped crystals with a length of 1–2.5 μm, and also amorphous precipitation was detected in all preparations. HPLC analyses of the supernatants revealed that more than 99% of GLP-1 was precipitated.

3.3. The solution and the suspension of crystals containing protamine sulphate

The plasma concentration profiles are shown in Fig. 4. The individual plasma concentration profiles, but not the mean, showed signs of a two phase absorption after injection of the protamine GLP-1 suspension. $t_{1/2}$ could not be calculated after injection of the protamine GLP-1 suspension because the elimination phase could not be detected within 15 h. The plasma half-lives obtained after injection of the protamine GLP-1 solution did not equal the $t_{1/2\lambda 1}$ and $t_{1/2}$ from the i.v. study but it equals the $t_{1/2}$ from the s.c. study with a GLP-1 solution. The plasma concentration of GLP-1 was increased above a pharmacological level from 0.5 to 10 h and to a pharmacological level from 0.5 to 15 h after administration of the protamine GLP-1 solution or the protamine GLP-1 suspension, respectively. This suspension also consisted of needle-shaped crystals together with amorphous precipitation, the length of the crystals was 5–6.5 μm.

4. Discussion

The plasma concentration of GLP-1 could only be followed for 15 h, because the dogs were fed after 15 h. GLP-1 is an endogenous hormone, secreted from the distal ileum as a response to an oral intake of food (Ørskov, 1992). Thus, after a meal the plasma concentration of endogenous GLP-1 could not be distinct from exogenous GLP-1 and would, accordingly, interfere with the pharmacokinetic analyses.

Three of the preparations, the GLP-1 solution, the GLP-1 suspension and the protamine GLP-1 suspension, gave almost identical plasma concentration profiles and, no significant differences were

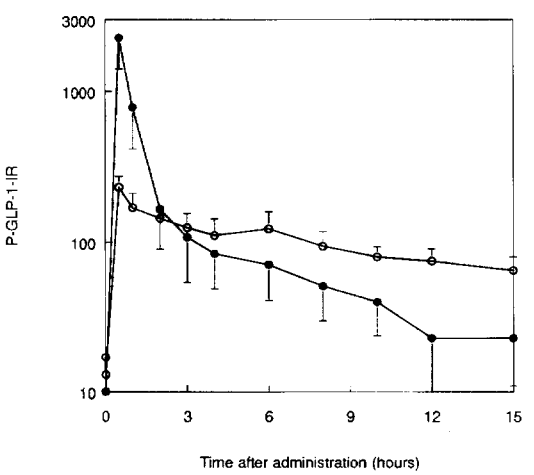

Fig. 4. Plasma GLP-1 immunoreactivity (pmol/l) in Beagle dogs following a single subcutaneous injection of GLP-1 50 μg/kg, as a solution (closed circles) or as a suspension of crystals (open circles) containing 0.5 mg/ml of protamine sulphate. $N = 4$. Data are mean ± S.E.M.

detected. The apparent half-life after s.c. administration was considerably longer than the half-life after i.v. administration. Hence, the absorption rate was the rate limiting step and the half-life after s.c. administration reflects the absorption rate.

Following s.c. administration of zinc or protamine GLP-1 suspensions, the plasma concentration profiles from all four preparations were similar. They all showed signs of two phase absorption, and no significant elimination phase could be detected within the duration of the study (15 h). From 6 h post-dosing to the end of the study a more or less stable plateau was reached. The mean increment in P-GLP-1-IR during the period from 6 to 15 h post dosing was 65 ± 17, 66 ± 19, 39 ± 9 and 72 ± 21 pmol/l following administration of the preparation containing 0.5, 1 and 3 equiv. zinc chloride and 0.5 mg/ml of protamine sulphate, respectively. The initial peak represented by C_{max} was elevated compared to the plateau level by a factor of 2.6 ± 0.5, 3.4 ± 0.6, 2.2 ± 0.3 and 3.7 ± 1.2 following administration of the preparation containing 0.5, 1 and 3 equiv. zinc chloride and 0.5 mg/ml of protamine sulphate, respectively. The cause of two-phase absorption in the crystalline preparations containing either zinc chloride or protamine sulphate has not yet been identified. However, as less than 1% of the GLP-1 was dissolved in the zinc or protamine GLP-1 suspensions, it is not likely that the initial peak could be explained by a quick absorption of the dissolved GLP-1. Whether or not it was caused by the mixed structure of the precipitate, crystals mixed with amorphous particles, the amorphous GLP-1 being dissolved more easily than the crystalline GLP-1 giving rise to the first phase in the absorption needs further investigation. The more or less stable plateau was clearly caused by a zero-order absorption in the second absorption phase. Accordingly, the absorption rate was significantly decreased and constant, when the suspension contained either zinc chloride or protamine sulphate compared to both a GLP-1 solution without or with protamine sulphate and a GLP-1 suspension. As no elimination phase was detected, the AUC could only be calculated from 0–15 h, no extrapolation to estimate the AUC from 0 to ∞ could be performed. Thus, the total AUC and with it, the value of f, was larger than given in Table 2, but it is impossible from this study to tell by how much. The most stable plasma concentration profile with the least fluctuations arose from the preparation with 3 equiv. zinc chloride.

The plasma concentration profile after s.c. administration of a GLP-1 solution would not be suited for clinical use due to the high peak level. Accordingly, if the dose was adjusted so that the initial peak plasma concentration of GLP-1 was within the desired pharmacological level, a pharmacological level could only be maintained for 1–3 h. In contrast, the plasma concentration profile after administering the peptide s.c. as either zinc or protamine GLP-1 suspensions might possibly be suited for clinical use, since the peak plasma concentration was flattened out so that the plasma concentration profile was close to being without a peak value.

In conclusion, the bioavailability varied between 20–50%, when the peptide was administered s.c. as a GLP-1 solution or a GLP-1 suspension and above 5–15% when administered s.c. as a zinc or protamine suspension. The absorption rate was decreased when the GLP-1 suspension contained either 0.5, 1 or 3 equiv. zinc chloride (relative to GLP-1) or 0.5 mg protamine sulphate/mg GLP-1 compared to a GLP-1 solution. The plasma concentration of GLP-1 achieved was increased above a pharmacological level from 0.5 to 10 h after administration of the peptide s.c. as a solution or a suspension and to a pharmacological level for at least 15 h when given as a zinc or protamine suspension. Furthermore, when administering the peptide s.c. as a zinc or protamine suspension the peak plasma concentration was flattened out. These results indicate that a formulation suitable for therapeutic use can be developed using these well-established protraction principles.

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