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Single- and multiple-dose pharmacokinetics of exendin-4 in rhesus monkeys

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Abstract

A radioimmunoassay (RIA) for the measurement of exendin-4 concentration in rhesus monkeys serum was developed and validated. The radioimmunoassay described here was sensitive, linear, accurate, precise, and reproducible. Range of the assay was 25–2000 pg/ml. Using this method we characterized the pharmacokinetics and accumulation of exendin-4 in rhesus monkeys. Following s.c. administration at doses rate of 1, 3 and 10 μg/kg, average C_{max} ranged from 2.26 ± 0.15 to 22.72 ± 1.54 ng/ml, and $AUC_{0-\infty}$ ranged from 3.43 ± 0.05 to 47.1 ± 0.10 ng h/ml. As compared to the i.v. administration at a single dose of 3 μg/kg, the absolute bioavailability after s.c. administration were estimated to be 67.3 ± 5.3, 75.1 ± 4.7 and 72.7 ± 8.4% for 1, 3 and 10 μg/kg dose, respectively. After daily s.c. administration at 10 μg/kg for 7 consecutive days, the accumulation ratio was approximately to 1.0, indicating no accumulation upon multiple doses in the monkeys.
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Keywords: Radioimmunoassay; Magnetic beads; Exendin-4; Pharmacokinetics; Rhesus monkeys

1. Introduction

Diabetes mellitus (DM) has reached epidemic proportions and numerous reports have documented the sharply increasing incidence of DM in the world (Zimmet et al., 2001; Lenhard and Gottschalk, 2002; Wild et al., 2004). In 2003, the International Diabetes Federation (IDF) estimated that almost 200 million people around the world had DM. By 2025 this figure is expected to rise to 333 million, amounting to 6.3% of the world's population living with DM. Approximately 90–95% of people with DM have type 2 diabetes mellitus (T2DM), which is characterized by hyperglycemia, insulin resistance, absolute or relative insulin deficiency, hyperglucagonemia and increased hepatic glucose production (Gadsby, 2002; Knowler et al., 2002; Leahy, 2005). Control of circulating glucose levels is rarely optimal, and many currently available therapies also have unfavorable side effects and restrictions, limiting the extent of their use (DeFronzo, 2004). Thus, there is an imperative need for novel therapeutic approaches for glycemic control that can complement existing therapies and possibly attempt to preserve normal physiological response to meal intake. One such approach is based on the action of the glucagon-like peptide 1 (GLP-1). GLP-1 is an incretin hormone, which is released from the L cells of the distal intestine in response to nutrients. It possesses a number of beneficial antidiabetic properties, such as glucose-dependent enhancement of insulin secretion, glucose-dependent suppression of inappropriately high glucagon secretion, slowing of gastric emptying, reduction of food intake and body weight. It may even promote β-cell preservation and improved neogenesis (Perry and Greig, 2003; Takei and Kasatani, 2004; Arulmozhia and Portha, 2006). Unfortunately, the usefulness of the native peptide is limited by its metabolic instability. Circulating GLP-1 undergoes rapid proteolytic cleavage by dipeptidyl peptidase-IV (DPP-IV), a ubiquitous serine protease, and biologically active GLP-1 has an apparent serum half-life of only 1–2 min in humans (Deacon

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et al., 1998). Given the rapid inactivation and short half-life of GLP-1, long acting agonists of the GLP-1 receptor have been developed.

Exendin-4, a 39-amino acid peptide that shares 53% amino acid sequence homology with GLP-1, was originally extracted from the venom of the *Heloderma suspectum* lizard monster (Eng et al., 1992; Goke et al., 1993). Exendin-4 has been shown in a variety of animal and cell models as well as in humans to share the same glucoregulatory actions of GLP-1 (Edwards et al., 2001; Egan et al., 2002; Meneilly et al., 2003; Nielsen et al., 2004; Michael and Juris, 2005; Iltz et al., 2006). Moreover, the actions of exendin-4 were also potentiated by its resistance to cleavage by DPP-IV, resulting in a longer half-life and duration of action and greater potency *in vivo* (Deacon et al., 1998). Based on the results of preclinical and clinical trials, Byetta® (Exenatide), a synthetic version of exendin-4, was approved by the U.S. Food and Drug Administration in April 2005 for adjunctive glycemic control in patients with T2DM who are taking metformin, a sulfonylurea, or a combination of metformin and a sulfonylurea (Yoo et al., 2006).

Previous studies have investigated the pharmacokinetics of exendin-4 following different administration routes in rats (Young et al., 1999; Parkes et al., 2001) and humans (Meneilly et al., 2003; Federico et al., 2005; Kolterman et al., 2005; Soon et al., 2006; Linnebjerg et al., 2007). In these preclinical and clinical trials, serum exendin-4 concentrations were mostly measured by Amylin Pharmaceuticals, Inc. (San Diego, USA) using an immunoenzymetric assay (IEMA) (Petrella et al., 2001). In this paper, we developed a radioimmunoassay (RIA) for exendin-4 with the aid of the magnetic separation system. Using this method we characterized the pharmacokinetics of exendin-4 following single and repeated s.c. administration in rhesus monkeys. To our knowledge, the pharmacokinetics of exendin-4 in nonhumans' primates has not been reported. This is the first report to evaluate the pharmacokinetics of exendin-4 in rhesus monkeys.

2. Materials and methods

2.1. Materials

The tested exendin-4 (with amino acid sequence of HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2, 4186.7 Da) was provided by Baolijian Genetic Engineering Co. Ltd. (Dongguan, China). The drug was available as white lyophilized powder and the purity was proved to be greater than 95.0% by high-performance liquid chromatography (HPLC). An artificial peptide (Tyr-exendin-4) was designed to consist of the 39-amino acid peptide extended at the C terminus so as to contain a tyrosine residue for iodination and was prepared by CL. (XIAN) Bio-Scientific Co. Ltd. (Xian, China). The purified 40-amino acid peptide was 96.5% homogeneous as determined by HPLC and the mass spectrum (MS) analyses yielded a mass of 4349.6 Da for Tyr-exendin-4 (calculated MW = 4350.8 Da). $Na^{125}I$ (99.0% purity and 638.0 GBq/mg) was purchased from Amersham Biosciences Ltd. (Amersham, UK). The other common chemicals were provided by standard commercial sources and were of the highest quality available.

2.2. Animals

Rhesus monkeys and New Zealand white rabbits were supplied by the Animal Raising Center of the Academy of Military Medical Sciences. The animals were individually housed in stainless-steel cages in a room with controlled temperature (25 ± 1 °C) and humidity (55 ± 5%) and a 12-h light/dark cycle. The animals were fed with standard diet and had free access to water. All procedures involving animals and their care were carried out according to the guidelines of the Institutional Ethical Committee for Care and Use of Laboratory Animal of Academy of Military Medical Sciences in accordance with the governmental guidelines on animal experimentation, National Institutes of Health "Principles of Laboratory Animal Care".

2.3. Development of exendin-4 RIA

2.3.1. Immunization and preparation of antiserum

Immunisation was performed in two male New Zealand white rabbits (weighing 3.0 ± 0.2 kg). Exendin-4 (100 μg to 250 μl in physiological saline) was emulsified with an equal volume of Freund's complete adjuvant (Sigma, USA). This suspension was injected into ear vein. Booster injections of 100 μg of immunogen were given every 4 weeks as an emulsion in Freund's incomplete adjuvant (Sigma, USA). Blood samples were collected from the marginal ear vein 7–10 days after each booster injection, and the titer of the antiserum determined. The rabbits were bled out under deep anaesthesia when the antibody titer reached a satisfactory level.

Purifications of antiserum on 1 ml protein A column (Amersham, UK) were carried out as recommended by the manufacturer's protocols. Antiserum from rabbits were diluted with tris(hydroxymethyl)aminomethane buffer (Tris buffer, pH 8.0) and loaded onto the protein A column. The column was subsequently washed with Tris buffer (pH 8.0) until OD_{280} returned to baseline and retained antibodies were eluted with 5 ml glycin buffer (50 mmol/l, pH 3.0). The eluted antibodies were immediately neutralized with Tris buffer (pH 8.0), rapidly frozen and stored at −20 °C prior to use.

2.3.2. Radioiodination of Tyr-exendin-4 and purification

A tracer was produced by iodination of Tyr-exendin-4 with ^{125}I, using the chloramine T method (Hunter and Greenwood, 1964). Briefly, 18.5 MBq of $Na^{125}I$ in 10 μl of 0.1 mol/l sodium hydroxide was added to a 1.5 ml plastic tube containing 2 μg of Tyr-exendin-4 in 50 μl PBS (pH 7.4) on ice. Then 25 μg of chloramine T (10 μl) solution in PBS (pH 7.4) was added. After 5 s of mixing, the mixture was incubated for another 25 s at room temperature. Iodination of the peptide was stopped by addition of 100 μl acetic acid buffer (0.1 mol/l, pH 4.0). The final mixture was loaded onto a 1 cm × 29 cm Sephadex G-15 column (Amersham, UK) for the separation of unincorporated free iodide from ^{125}I-labeled Tyr-exendin-4. The radioiodinated materials were eluted with acetic acid buffer (0.1 mol/l, pH 4.0),

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