Nuclear Medicine and Biology 43 (2016) 397–402

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

# Cardiovascular side-effects and insulin secretion after intravenous administration of radiolabeled Exendin-4 in pigs

Anneli Rydén [a,*], Görel Nyman [a], Lovisa Nalin [a], Susanne Andreasson [a], Olle Korsgren [b], Olof Eriksson [c], Marianne Jensen-Waern [a]

[a] Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
[c] Department of Medicinal Chemistry, Preclinical PET Platform, Uppsala University, Uppsala, Sweden

ARTICLE INFO

*Article history:*
Received 20 November 2015
Received in revised form 27 March 2016
Accepted 14 April 2016

*Keywords:*
Exenatide
Adverse-effects
Tachycardia
Arrhythmias
Swine
Imaging
PET
Monkeys

ABSTRACT

*Introduction:* Radiolabeled Exendin-4, a synthetic glucagon-like peptide-1 (GLP-1) analog, is used as a tracer for diagnostic purposes of β-cells and in experimental animal research. Exendin-4 can be radiolabeled with $^{68}$Ga, $^{111}$In or $^{99m}$Tc and used for positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging to diagnose insulinomas, visualization of pancreatic β-cell mass and transplanted Islets of Langerhans. In humans, Exendin-4 is widely used as a therapeutic agent for treatment of type 2 diabetes (T2D). The compound, which is administered subcutaneously (SC) may cause nausea, vomiting and a minor increase in the heart rate (HR). However, possible side-effects on cardiovascular functions after intravenous (IV) administration have not been reported. This study describes the Exendin-4 dose at which cardiovascular side-effects occur in pigs and cynomolgus monkeys. The IV effect of the tracer on insulin secretion is also investigated in pigs.

*Methods:* Seven clinically healthy littermate pigs (40 days old) were used; three of them were made diabetic by streptozotocin (STZ). All pigs underwent PET imaging under general anesthesia to examine the glucagon-like peptide-1 receptor (GLP-1R) in β-cells with radiolabeled Exendin-4. A baseline tracer dose IV [$^{68}$Ga]Exendin-4 (0.025 ± 0.010 μg/kg) followed by a competition dose IV [$^{68}$Ga]Exendin-4 (3.98 ± 1.33 μg/kg) 60 min later were administered. Blood samples were taken and analyzed for insulin secretion by using ELISA. Cardiovascular and respiratory variables were monitored throughout the experiment.

*Results:* Immediately after administration of the high dose [$^{68}$Ga]Exendin-4 the HR rose from 122 ± 14 to 227 ± 40 bpm ($p < 0.01$) and from 100 ± 5 to 181 ± 13 bpm ($p < 0.01$) in healthy non-diabetic and diabetes-induced pigs, respectively. The tachycardia was observed for >2 h and one healthy non-diabetic pig suffered cardiac arrest 3 h after the IV [$^{68}$Ga]Exendin-4. Arrhythmia was detected by listening to the heart with a stethoscope up to 4 days after the [$^{68}$Ga]Exendin-4 injection. In all animals, no effect on the cardiovascular system was registered after the low dose of IV [$^{68}$Ga]Exendin-4. Insulin secretion increased ($p < 0.05$) when IV [$^{68}$Ga]Exendin-4 was given in dosages ≥0.14 μg/kg.

*Conclusions:* Intravenous administration of ≥2.8 μg/kg [$^{68}$Ga]Exendin-4 resulted in severe tachycardia and arrhythmias in healthy non-diabetic and diabetes-induced pigs, and the insulin secretion was stimulated in healthy non-diabetic animals when ≥0.14 μg/kg [$^{68}$Ga]Exendin-4 was given.



## 1. Introduction

Glucagon-like peptide-1 (GLP-1) is a neuropeptide and promotes the synthesis of insulin, increases glucose-dependent insulin secretion and improves β-cells responsiveness to glucose [1]. The glucagon like peptide-1 receptor (GLP-1R) is highly expressed on β-cells [2,3] and plays an important role in glucose homeostasis. Thus GLP-1R agonists, such as synthetic Exendin-4 (exenatide), improve glucose control by upregulating insulin, and are widely used in treatment of type 2 diabetes (T2D). Exenatide is injected subcutaneously (SC) as a short acting form (Byetta®, AstraZeneca, Sweden) or as a long acting form (Bydureon®, AstraZeneca, Sweden) [4–6]. The most common side-effects reported of SC exenatide arise from the gastrointestinal tract but a meta-analysis by Robinson et al. [7] also reports a small rise in heart rate (HR). The GLP-1R is overexpressed in insulinomas [8], and the receptor is regarded as a promising target for radionuclide imaging. In humans a receptor specific ligand, Exendin-4 radiolabeled with $^{68}$Ga has been injected intravenously (IV) using positron emission tomography (PET) to detect these tumors [9], or radiolabeled with $^{111}$In or $^{99m}$Tc

* Corresponding author at: Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, PO Box 7054, S-750 07, Uppsala, Sweden. Tel.: +46 18 671890.
*E-mail address:* anneli.ryden@slu.se (A. Rydén).

http://dx.doi.org/10.1016/j.nucmedbio.2016.04.002
0969-8051/

**Table 1**
Overview of reported side-effects from 80 patients after IV administration of radiolabeled Exendin-4. Areas: oncology (insulinoma, adult nesidioblastosis) and diabetes (T1D, intraportally and intramuscularly transplanted islets).

| Type of tracer | Patient population | Number of patients | Reference | Dose | Side-effects |
|---|---|---|---|---|---|
| $^{111}$In-DTPA-Exendin-4 | Insulinoma | 1 | N Engl J Med 359;7 August 14, 2008 | Not available | No comment |
| $^{111}$In-DOTA-Exendin-4 | Insulinoma | 6 | J Clin Endocrinol Metab 2009 Nov.; 94(11):4398–4405 | 30 μg | Vomiting No further side-effect |
| $^{111}$In-DTPA-Exendin-4 | Transplanted T1D | 1 | N Engl J Med 363;13 September 23, 2010 | Not available | No comment |
| $^{111}$In-DTPA-Exendin-4 | Insulinoma | 11 | J Nucl Med 2011; 52:1073–1078 | 20 μg | Lowered blood glucose |
| 99Tc-HYNIC-Exendin-4 | Insulinoma | 11 | Eur J Nucl Med Mol Imaging. 2013 Apr.; 40(4):524–31 | 30 μg | No adverse reactions |
| $^{111}$In-DTPA-Exendin-4 | Insulinoma | 30 | Lancet Diabetes Endocrinol. 2013 Oct.;1(2):115–22 | 8–14 μg | Lowered blood glucose |
| $^{111}$In-DTPA-Exendin-4 | T1D and healthy controls | 5 + 5 | Diabetologia. 2014 May; 57(5):950–9 | 1 μg | No comment |
| $^{68}$Ga-DOTA-Exendin-4 | Insulinoma | 1 | J Clin Endocrinol Metab. 2014 May; 99(5):1519–24 | 13 μg | No adverse reactions |
| $^{68}$Ga-DOTA-Exendin-4 | Adult nesidioblastosis | 1 | Endocrine. 2015 May 23 | Not available | No comment |
| $^{111}$In-DOTA-Exendin-4 | Insulinoma | 3 | Patient Saf Surg. 2015 Jun. 2;9:2 | Not available | No comment |
| $^{111}$In-DOTA-Exendin-4 | Insulinoma | 5 | J Nucl Med. 2015 Jul.;56(7):1075–8 | 12–15.3 μg | Nausea |

for single-photon emission computed tomography (SPECT) imaging [10,11]. Furthermore, $^{111}$In Exendin-4 has been used in human research for imaging of the β-cell mass in pancreas [12] and transplanted Islets of Langerhans [13]. The side-effects reported, *i.e.* nausea, vomiting and lowered blood glucose, after IV administration are summarized in Table 1.

In a previous unpublished experiment we injected exenatide in four cynomolgus monkeys. Tachycardia developed in all animals but whether the cause of tachycardia was due to IV exenatide, was not further investigated. Therefore, we examined the possible cardiovascular side-effects of IV [$^{68}$Ga]Exendin-4 in pigs. The present experiment involves both healthy non-diabetic and streptozotocin (STZ) diabetes-induced pigs. In addition to selectively destroy the β-cells in pancreas, STZ is known to be nephrotoxic and hepatotoxic [14,15]. However, in accordance with earlier studies [16,17] no toxic effects were observed in the present study when diabetes was induced in pigs with IV 150 mg/kg STZ. [$^{68}$Ga]Exendin-4 was given as a tracer and cardiovascular and respiratory variables were continuously monitored for three hours. Auscultation (examination by listening with a stethoscope) of the heart and lungs was performed five times every day up to four days after IV [$^{68}$Ga]Exendin-4 administration. Since another possible side-effect after IV administration of the compound is hypoglycemia (see Table 1), the effect of exenatide on the insulin secretion was analyzed in normal pigs. Severe cardiovascular effects, such as prolonged tachycardia, were recorded immediately after IV injection of exenatide. These changes and the effect of IV [$^{68}$Ga]Exendin-4 on insulin serum concentrations in pigs are reported in the present study.

## 2. Methods and materials

### 2.1. Animals

Seven 40 days old high health herd certified littermate pigs of both sexes (Yorkshire × Landrace × Hampshire) were obtained from the university farm. The pigs were randomly divided into two groups: controls, n = 4 (No. 2, 4, 6 and 7) and principals, n = 3 (No. 1, 3 and 8). After the main study an additional healthy pig (aged 120 days) from the same herd was provided for brain scanning. The pigs were housed individually at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, in pens measuring approximately 3 m$^2$. Straw and wood shavings were used as bedding and all pigs had an infrared heat lamp in the pen. A light/dark schedule 12:12 h was used and the temperature in the room was controlled (18 ± 2 °C). The pigs were fed a commercial diet for growing pigs without growth promoters and antimicrobials twice daily (SOLO 330 P SK, Lantmännen, Sweden). Water was provided *ad libitum*.

During a 14 day acclimatization period the pigs were tamed and handled. Throughout the study, the pigs were clinically examined daily by an experienced veterinarian. To allow blood sampling and IV injections, an indwelling silicon catheter (SIL-C70 with rounded tip; Instech Solomon, Plymouth Meeting, PA, USA) was placed in the jugular vein under general anesthesia and aseptic conditions, according to Jensen-Waern et al. [16]. During the anesthesia, the three principals were given 150 mg/kg STZ (Sigma S0130, Stockholm, Sweden) IV. STZ is toxic to β-cells thus the pancreatic β-cells were directly ablated. Hyperglycemia (>24 mmol/L) was induced within 24 h, and clinical signs of diabetes developed subsequently, for details see Nalin et al. [3].

Seven days after IV STZ, insulin treatment with an intermediate-acting porcine insulin (Caninsulin vet. 40 IU/mL, Intervet AB, Sollentuna, Sweden) was given SC twice daily at a starting dose of 0.8 IU/kg. Dosages were adjusted several times throughout the day in response to blood glucose level.

In general, the pig is widely used as a large animal model because of its anatomical and physiological similarities to humans. Further, the pig is used in diabetes research since diabetes can be readily induced with STZ. The metabolic changes are in accordance with those seen in man [16].

All procedures were approved by the Ethics Committee for Animal Experimentation, Uppsala, Sweden. All applicable international, national, and institutional guidelines for the care and use of animals were followed. In total, the protocol ran for 60 days.

### 2.2. Imaging sessions

The PET imaging was performed at the Uppsala University Hospital and the pigs were anesthetized before transportation (15 min by car) from their home pens. Anesthesia is necessary for a smooth transportation, catheterization, blood sampling and immobility in dorsal recumbency during scanning. Feed, but not water, was withheld 8 h prior to anesthesia. Two different anesthetic protocols were used. The healthy non-diabetic pigs were anesthetized according to our regular anesthetic protocol for healthy pigs, *i.e.* 5 mg/kg tiletamine and zolazepam (Zoletil Forte® vet. 250 mg/mL, Virbac, Carros, France) combined with 0.05 mg/kg medetomidine (Domitor® vet 1 mg/mL, Orion Pharma Animal Health, Sollentuna, Sweden) and 0.1 mg/kg butorphanol (Dolorex® vet. 10 mg/mL, Intervet AB, Sweden) intramuscularly (IM). The anesthesia was maintained by a constant rate infusion of 5 mg/kg/h tiletamine and zolazepam, combined with 0.05 mg/kg/h medetomidine, and 0.1 mg/kg/h butorphanol IV. In the diabetes-induced group, anesthesia was induced with 4 mg/kg alfaxalone (Alfaxan®10 mg/mL, Vétoquinol UK Limited, UK), 2 mg/kg midazolam (Dormicum® 5 mg/mL, Roche AB, Sweden) and 2 μg/kg fentanyl (Fentanyl B® Braun 50 μg/mL, B. Braun Medical AB, Sweden) IV. The anesthesia was maintained with an infusion, starting at a dose of 3 mg/kg/h alfaxalone, 1 mg/kg/h midazolam and 1 μg/kg/h fentanyl, continuously adjusted to achieve a light plane of anesthesia. The protocol for diabetes-induced pigs was chosen based on the fact that the alfaxalone is short-acting, presumed to have a minimal impact on the pre-existing pathological conditions. No paralyzing muscle relaxants were administered in any of the groups. During the PET imaging the pigs were