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ORIGINAL ARTICLE

Glucagon-like peptide-1 receptor imaging with [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 for the detection of insulinoma

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Abstract
Purpose The objective of this article is to present a new method for the diagnosis of insulinoma with the use of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4.
Methods Studies were performed in 11 patients with negative results of all available non-isotopic diagnostic methods (8 with symptoms of insulinoma, 2 with malignant insulinoma and 1 with nesidioblastosis). In all patients glucagon-like peptide-1 (GLP-1) receptor imaging (whole-body and single photon emission computed tomography/CT examinations) after the injection of 740 MBq of the tracer was performed.
Results Both sensitivity and specificity of GLP-1 receptor imaging were assessed to be 100 % in patients with benign insulinoma. In all eight cases with suspicion of insulinoma a focal uptake in the pancreas was found. In six patients surgical excision of the tumour was performed (type G1 tumours were confirmed histopathologically). In one patient surgical treatment is planned. One patient was disqualified from surgery. In one case with malignant insulinoma pathological accumulation of the tracer was found only in the region of local recurrence. The GLP-1 study was negative in the other malignant insulinoma patient. In one case with suspicion of nesidioblastosis, a focal accumulation of the tracer was observed and histopathology revealed coexistence of insulinoma and nesidioblastosis.
Conclusion [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 seems to be a promising diagnostic tool in the localization of small insulinoma tumours, but requires verification in a larger series of patients.

Keywords Insulinoma · GLP-1 · Imaging · Labelled peptides

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Introduction

Overexpression of somatostatin receptors on the neuroendocrine tumour cell surface is a very well-known phenomenon, but not all neoplasms demonstrate a high incidence of this receptor subtype. Therefore, there is a need to search for other peptide analogues for diagnostic and therapeutic purposes.

Insulinoma is the most common hormone-secreting neoplasm localized in the pancreas. In the majority of cases the tumours are benign. Their malignant form is observed in less than 10.0 % of insulinoma patients. Most often insulinoma appears as a singular lesion located in the pancreatic parenchyma (97.0 %), but rarely it might also be multifocal [1]. A clinically accepted method for the diagnosis of insulinoma is the positive result of a 72-h fasting test with a high insulin level (over 6.0 μIU/ml), elevated C-peptide level (over 200.0 pmol/l) and absence of sulfonylurea in the plasma [2]. Similar results also occur in cases of nesidioblastosis [1]. Due to the usually small sizes of insulinoma lesions and the technical limitations of the majority of standard diagnostic modalities, its anatomic localization is sometimes difficult. In some cases not only multiphase contrast-enhanced computer tomography (CT), magnetic resonance imaging (MRI) or standard ultrasound (US), but even endoscopic ultrasound (EUS) is not sensitive enough to visualize the tumour. The sensitivity of EUS for the detection of insulinoma lesions in the pancreatic head, body and tail was reported to be 92.6, 78.9 and 40.0 %, respectively [3]. However, other authors reported a higher sensitivity and specificity for the localization of intrapancreatic lesions by EUS (93.0 and 95.0 %, respectively) [4]. Techniques such as selective arteriography, transhepatic peripancreatic venous blood sampling (TPVB), intra-arterial calcium stimulation test (ASVS) and intraoperative US revealed better sensitivity (95.0 %), but are much more time-consuming and of course invasive [5]. Nuclear medicine imaging, such as somatostatin receptor scintigraphy (SRS), is positive in about 50.0–60.0 % of benign insulinomas [1, 2]. Surgery remains the only method for full recovery of insulinoma patients, but requires precise location of neoplasm foci. After a successful surgical therapy, a very good long-term survival outcome is being observed in patients with insulinoma [2, 6]. For these reasons more sensitive diagnostic options are required.

Glucagon-like peptide-1 (GLP-1) receptors have been found in normal organs such as pancreas, blood vessels, stomach or parafollicular C cells. GLP-1 receptor expression is also observed on different types of neoplastic cells (e.g. benign insulinoma, phaeochromocytoma, gastrinoma, paraganglioma, pulmonary neuroendocrine tumours and medullary thyroid carcinoma). According to Reubi and co-workers the density of GLP-1 receptors is extremely high especially on benign insulinoma cell surfaces. They have been found in almost 100 % of insulinoma cases—in contradistinction to ileal carcinoids, where GLP-1 receptor expression was found only in one third of cases with a heterogeneous distribution. Somatostatin receptors were detected in two thirds of examined insulinomas (mainly SSTR1, SSTR2 and SSTR5) [7]. Comparable results were reported by Bertherat et al. [8]. Therefore, in the near future GLP-1 receptor imaging may become a preferred diagnostic method for the localization of insulinomas hardly detectable by other examinations [7].

The first study with a labelled GLP-1 analogue {[Lys^{40}(Ahx-DTPA-^{111}In)NH$_2$]-exendin-4} was successfully performed by Wild et al. in two patients with insulinoma [9]. Exendin-4 is a 39-amino acid peptide hormone originally found in the saliva of the Gila monster, which has been proved to be a long-acting potent agonist for GLP-1 receptors. A subsequent clinical study with the same ^{111}In-labelled GLP-1 receptor agonist confirmed the high sensitivity of this method. In all examined patients benign insulinomas were detected (six of six) [10]. The same group of scientists used [Lys^{40}(Ahx-HYNIC/EDDA)NH$_2$]-exendin-4 labelled with ^{99m}Tc for the first time in a mouse model [11]. The use of ^{99m}Tc may improve the quality of images and radiation safety for patients and the staff by many procedural advantages related to the physical properties of this isotope.

The aim of this paper is to evaluate the clinical usefulness of the new radiopharmaceutical [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 injected into patients with suspicion of benign insulinomas that are hardly detectable or not diagnosed by other available methods.

Materials and methods

Patients

Eleven patients (seven women and four men, mean age 48.0 ±18.9 years, min. 16.0 years, max. 75.0 years) were enrolled in this study. There were eight patients with clinical and biochemical symptoms and signs of insulinoma, one patient with malignant insulinoma, one patient with suspected local recurrence of malignant insulinoma (MEN1) and one with nesidioblastosis. All patients were verified for a positive fasting test, elevated serum insulin and C-peptide level concentrations (fasting glycaemia 33.0–54.0 mg/dl, fasting insulinaemia 10.6–51.6 μIU/ml, C-peptide concentrations 4.7–6.3 ng/ml). None of the patients was treated with oral antidiabetic agents. Symptoms of hypoglycaemia have been observed for at least 1 year even up to 15 years in this group of patients. The Department of Endocrinology, Jagiellonian University Medical College in Cracow, Poland annually diagnoses up to 30 patients with suspicion of insulinoma. Because of difficulties in localizing the insulinoma focus by standard diagnostic methods, five to six of them were scheduled for the study with labelled GLP-1 analogue annually. Patients from other sites in Poland have also been diagnosed in our department during the time when this study was conducted.

Before GLP-1 receptor imaging, CT/MRI/EUS was performed in all patients; eight patients underwent SRS after

the injection of ^{99m}Tc-EDDA/HYNIC-TOC (Tektrotyd, Polatom, Poland) and two patients positron emission tomography (PET)/CT with ^{68}Ga-DOTATATE. In all patients with suspicion of benign insulinoma all these scans were negative or equivocal. It should be stated that in this group of patients the negative or equivocal result of previous diagnostic studies was one of two main inclusion criteria to perform the GLP-1 examination (after stated clinical symptoms of insulinoma). In the first patient with malignant insulinoma the CT was positive in liver metastasis, but negative in the site of recurrence. In the second patient with malignant insulinoma ^{68}Ga-DOTATATE PET/CT was positive for liver and lymph node metastases, but negative for the local recurrence. Detailed information about the patients is presented in Table 1.

After receiving detailed information about the study procedure, all patients gave their written consent. The study was approved by the local Ethics Committee.

Patients were placed on a liquid diet 1 day before the beginning of the examination and fasted on the day of the injection of the tracer. Each of them was carefully checked for any adverse reactions. Because of the natural disease course of insulinoma in fasting patients, blood pressure and glucose values were monitored before and after injection of the compound at several time points. Some of the patients required glucose infusion at the time of the GLP-1 receptor imaging procedure, because their level of glycaemia dropped below 40 mg/dl.

After GLP-1 receptor imaging patients without contraindications qualified for a surgical treatment. The histopathological confirmation of tumour presence and evaluation of its type was performed after surgery.

Preparation of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4

The radiolabelling of [Lys^{40}(Ahx-HYNIC)NH$_2$]-exendin-4 with ^{99m}Tc followed a two-vial kit formulation as previously published [11] with some modifications [12]. Briefly, 1 ml of a solution containing 30 mg (168 μmol) of tricine, 30 μg of [Lys^{40}(Ahx-HYNIC)NH$_2$]-exendin-4 and 33.6 μg of $SnCl_2$ (12 μl of 22.2 mM $SnCl_2 \times 2\ H_2O$ in 0.1 M HCl) was filtered into a glass vial strictly under air protection, and 1 ml of a solution containing 10 mg of EDDA (pH was adjusted to 7 with 1 M NaOH) was filtered into a second glass vial. The glass vials were immediately frozen in liquid nitrogen, lyophilized and closed afterward under nitrogen. For labelling, the EDDA vial was reconstituted with 1 ml of saline and 0.5 ml of it added to the [Lys^{40}(Ahx-HYNIC)NH$_2$]-exendin-4 vial, followed by 740–1,850 MBq of $^{99m}TcO_4^-$ in 0.3–1.5 ml eluate of ^{99}Mo/^{99m}Tc generator (Polatom, Poland) and incubated for 10 min at 100 °C. After cooling to room temperature, the reaction mixture was analysed by HPLC and thin-layer chromatography (TLC). The average radiochemical purity was more than 90 %. The mean injection activity for patients was 740 MBq.

Imaging technique

GLP-1 receptor imaging with the use of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 was performed at the Nuclear Medicine Unit of the Endocrinology Department, University Hospital in Cracow. Firstly, images were acquired with a dual-head, large field of view E.CAM gamma camera with low-energy high-resolution (LEHR) collimators. From

Table 1 Information about patients and examinations performed

Initials	Age	Sex	Diagnosis	Hypoglycaemia	SRS	CT	GLP-1	Subsequent treatment
J.A.	57	F	Ins. susp.	12 years	−	−	+	Surgery
R.G.	54	M	Ins. susp.	1 year	−	−	+	Qualified for surgery
J.W.	16	M	Ins. susp.	1.5 years	−	+/−	+	Surgery
A.P.	75	M	Ins. susp.	4 years	+	−	+	Disqualified from surgery
B.J.	38	F	Ins. susp.	10 years	^{68}Ga-DOTATATE −	−	+	Surgery
K.S.	52	F	Ins. susp.	NA	−		+	Surgery
M.K.	62	F	Ins. susp.	NA	Not done	−	+	Surgery
P.W.	21	M	Ins. susp.	NA	+	+/−	+	Surgery
A.K.	65	F	Mal. ins.	4 years	^{68}Ga-DOTATATE + in liver meta and lymph nodes	+ in liver meta, − in recurrence place	−	Pharmacotherapy
J.J.	57	F	Mal. ins.	15 years	−	+ in liver meta	− in liver meta, + in site of recurrence	Patient did not agree to surgery
A.U.	31	F	Nesidio	NA	+	+	+	Surgery

F female, *M* male, *NA* not available, − negative result, + positive result, +/− equivocal result, *Ins. susp.* suspicion of insulinoma, *Mal. ins.* malignant insulinoma, *Nesidio* nesidioblastosis

2010 onwards all examinations were performed with the use of the Symbia TruePoint T16 hybrid system, also with LEHR collimators (Siemens Healthcare).

On the basis of the first patients' examinations our intention was to find the optimal acquisition protocol and to perform dosimetric calculations. Due to this, initial whole-body scans were carried out at six time points, from the first hour and in some cases even up to 30 h (1, 2, 3, 6, 24 and 30 h) after the injection of the compound. Standard camera settings (with scan speed 12 cm/min for each examination) were used for whole-body examinations with ^{99m}Tc [13].

Diagnostic single photon emission computed tomography (SPECT) studies were performed at two time points, between 3 and 4 h and between 5 and 6 h after the injection of the tracer. The SPECT examinations were done with standard camera settings like those used in the SRS study after labelled somatostatin analogue injection [13]. The acquired data were reconstructed using the ordered subset expectation maximization (OSEM) FLASH 3-D iterative reconstruction method. After the installation of the new hybrid device in the Unit, SPECT/CT studies were carried out in all subsequent patients with the same settings for the SPECT part of the study and low-dose protocol for the CT part (130 kV, 13 mAs, slice thickness and reconstruction increment 5.0 mm).

In tumour tissue and kidneys, changes in the uptake were evaluated over time. Volumetric analysis was performed to assess the tumour to non-tumour ratio (T/nT ratio) and the kidney to non-tumour ratio (K/nT ratio). The total counts in defined regions of interest (ROI) were calculated. The background ROI was defined in the neighbouring normal tissue for each region. T/nT and K/nT ratios were assessed for SPECT scans performed between 2 and 3 h, 3 and 4 h, 5 and 6 h and 24 h (in two patients) after the injection of the compound. The obtained images were assessed by two experienced nuclear medicine specialists.

Absorbed dose assessment

We also analysed biokinetic characteristics of the new tracer and estimated the patient radiation dose. The organ radiation dose was assessed for five patients. The external conjugate view counting pair method was used for analysing whole-body images [14]. Three source organs were chosen: kidneys, liver and lungs. The area under the time-activity curve for the whole body as well as the biological half-time and the effective half-time for the tracer were estimated. The organ absorbed dose coefficient $D(r_T, T_D)$ was evaluated according to the Medical Internal Radiation Dose (MIRD) system [15][16]. The total effective dose, using weighting factors defined in International Commission on Radiological Protection (ICRP) publication 103, was also evaluated [17]. The blood clearance of the tracer and the radiation dose to the red marrow was established by the blood samples method [18]. The plasma and blood fractions were measured separately. Urine samples were also collected (after the 1 h scan and before 2 h, 3 h and 6 h scans). The total volume of excreted urine and its activity was measured after each collection.

Results

The quality of the obtained [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH_2]-exendin-4 images was evaluated as very good.

In eight of eight cases with suspicion of insulinoma, focal uptake of the tracer in the pancreas was found (Fig. 1). In two patients GLP-1 receptor scintigraphy was repeated because of uncertain localization of the tracer in the pancreas. The second study confirmed the previous foci localization. So far, successful surgical excision of the tumour has been performed in six patients. Post-surgical resolution of symptoms was observed in all patients. Histopathological studies confirmed type G1 neuroendocrine tumours. One patient is awaiting surgery. One patient was disqualified from surgery due to cardiological disorders. In one patient (38-year-old woman) histopathological examination revealed the coexistence of insulinoma and nesidioblastosis.

In the group of patients with benign insulinoma both sensitivity and specificity of GLP-1 receptor imaging were assessed as 100 %.

The first patient with confirmed malignant insulinoma (65-year-old woman) was operated in 2007 due to a tumour of the pancreatic tail. She was qualified for peptide receptor radionuclide therapy (PRRT) in 2008, because of inoperable liver metastases. A complete response to the therapy was observed. However, in 2010 the patient presented again with hypoglycaemia (below 35 mg/dl) and hyperinsulinaemia (up to 58.8 μIU/ml). PET imaging with ^{68}Ga-DOTATATE was positive for liver and lymph node metastases. The patient was also qualified for GLP-1 receptor imaging, which gave a negative result. The patient is being treated with verapamil and up to now symptoms of hypoglycaemia have not been observed any more. In the other patient (57-year-old woman), who has been suffering from MEN1 syndrome, the malignant insulinoma was resected in 1996. Liver and lymph node metastases were detected in 2010. GLP-1 receptor imaging confirmed the recurrence of malignant insulinoma (a focal uptake in the place of the removed pancreatic head), but uptake of neither GLP-1 nor somatostatin receptor analogues was observed in metastatic lesions. This patient did not agree to surgery.

Increased uptake of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH_2]-exendin-4 in the upper abdomen (the result similar to SRS) was seen in the case of a patient with nesidioblastosis (31-year-old woman) (Fig. 2). In this patient, after three operations, each with partial pancreas resection, the

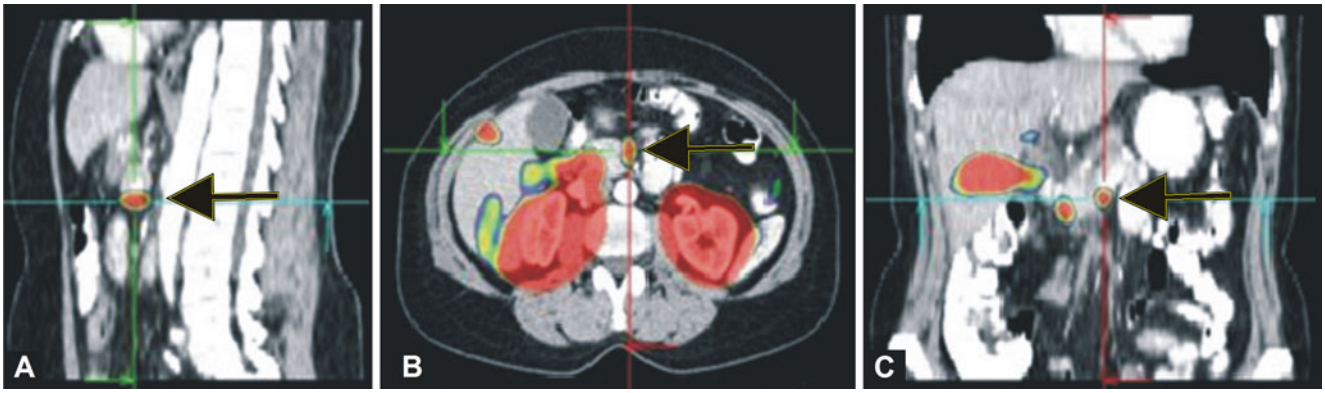

Fig. 1 Patient J.A. (57 years old) with clinically overt hypoglycaemia. The examination with the GLP-1 analogue labelled with ^{99m}Tc revealed focal accumulation of the tracer. The well-differentiated neuroendocrine tumour type G1 was found in the histopathological study after surgical excision of the part of the pancreas head selected during GLP-1 receptor imaging study. **a** Fusion of GLP-1 receptor imaging and CT sagittal slice. **b** Fusion of GLP-1 receptor imaging and CT axial slice. **c** Fusion of GLP-1 receptor imaging and CT coronal slice

observed focal accumulation of the tracer was necessary for further verification. The patient underwent surgical treatment, but died after the fourth surgery due to internal bleeding. The histopathological examination confirmed insulinoma coexisting with nesidioblastosis.

No adverse reactions such as nausea, decreased blood pressure, bronchospasm, bradycardia, skin flushing or itching, hives or other effects after the tracer injection were reported by any of the patients at the time of the examination. Generally, we did not observe exacerbation of hypoglycaemia related to tracer injection, but this was difficult to verify in every patient because of the natural disease course of insulinoma and the necessity of a constant glucose infusion in most cases to prevent the drop of glucose to levels which would be dangerous for the patient.

Changes in the average uptake in tumour tissue and kidneys over time for the examined group of patients are presented in Fig. 3.

As it was estimated for ^{111}In-DOTA-exendin-4, the curve for the plasma samples revealed a biexponential clearance (a very fast alpha phase $T_{1/2}$=12 min and a visibly slower beta phase $T_{1/2}$=1 h 57 min) [9]. A low activity concentration was also observed in blood samples, which probably was related to the noneffective centrifugation or the contamination of samples for the gamma counter measurements at the time of their collection. The cumulated activity in the total volume of excreted urine, which was measured up to 6 h post-injection, revealed that the saturation of the curve was not reached. Absorbed dose estimates are shown in Table 2.

Discussion

This paper presents our first experience with [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 in the detection of the pancreatic neuroendocrine tumour secreting insulin, insulinoma. To the best of our knowledge, it is the first application of this new compound in clinical practice. For most of the patients involved in this study, examination with this novel biomarker was the last hope for the localization of the insulinoma foci and further curative surgical treatment. We were able to detect tumour lesions (benign insulinoma foci) with an excellent sensitivity and specificity. The quality of images was evaluated as high by referring physicians.

Malignant insulinomas frequently lack GLP-1 receptors (positive GLP-1 receptor imaging only in 36 % of cases)

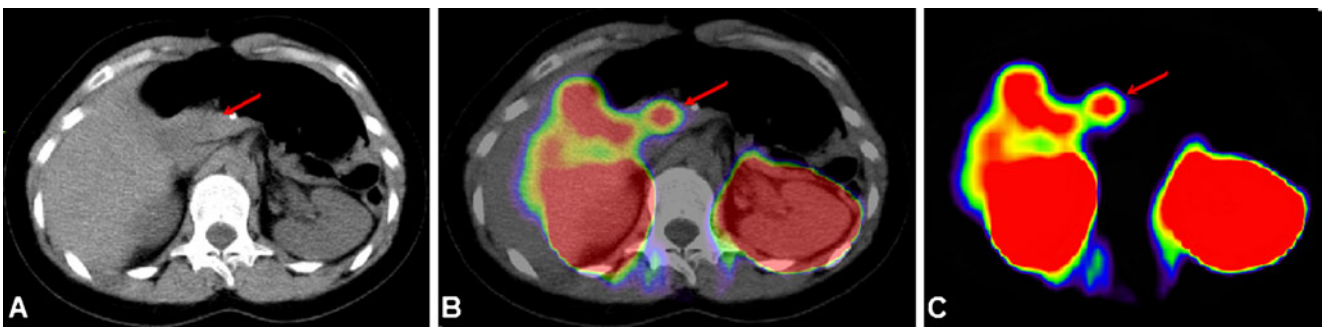

Fig. 2 Patient A.U. (31 years old) with nesidioblastosis. The examination with the GLP-1 analogue labelled with ^{99m}Tc revealed focal accumulation of the tracer in the pancreatic head. Insulinoma with coexisting nesidioblastosis was confirmed in the histopathological examination after surgery. **a** CT study. **b** Fusion of GLP-1 receptor imaging and CT. **c** GLP-1 receptor imaging

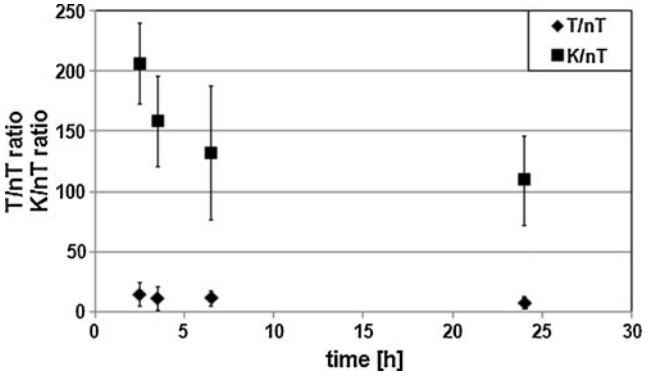

Fig. 3 Tumour to non-tumour ratio (*T/nT*) and kidney to non-tumour ratio (*K/nT*) over time (means ± SD). The T/nT remained relatively stable over time, while the K/nT decreased visibly (~ 40 % from the time of injection to 6 h after injection)

but, in contrast to benign insulinomas, more often express SST2 receptors (positive SRS scan in 73 % of cases) [19]. This finding is very interesting because it shows the ability of GLP-1 receptor imaging for predicting malignant or benign status of an insulinoma lesion. There were only two patients in our group with malignant insulinoma in whom GLP-1 scintigraphy was performed. In one of them, GLP-1 receptor imaging confirmed the local recurrence of malignant insulinoma, but no uptake of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 was observed in liver metastases. This case might suggest the different biology of primary lesions and their metastases in terms of the expression of GLP-1 receptors. This phenomenon is well known for somatostatin receptors in neuroendocrine tumours [20].

According to Wild et al., hypoglycaemia after ^{111}In-DTPA-exendin-4 (10±2 μg) injection is observed in patients with malignant insulinoma [19]. The decrease of blood glucose level was more often seen in cases of patients with GLP-1 receptor-positive lesions than in those with negative ones. The mechanism of this phenomenon is unclear, as is the mechanism of possibly more severe hypoglycaemia occurring after cold somatostatin analogue injection in patients with malignant insulinoma. It is probably caused by the inhibitory effect of the somatostatin analogue on glucagon excretion [21]. In the cases of insulinoma patients enrolled in our study it was difficult to assess the changes in glucose levels related only to the labelled GLP-1 injection because some of them received constant glucose infusion to prevent hypoglycaemia. Based on our experience, we believe that establishing this fact is a very difficult task, due to the natural disease course of insulinoma. Because of significant differences in the patients' conditions before the examination, each patient needed to be managed individually.

Table 2 Organ absorbed dose estimates for [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4

Organ	Mean estimated absorbed dose ± SD (mGy/MBq)
Kidneys	0.1124±0.0526
Liver	0.0055±0.0009
Lungs	0.0036±0.0007
Spleen	0.0052±0.0023
Pancreas	0.0043±0.0017
Intestine	0.0066±0.0026
Red marrow	0.0052±0.0010
Thyroid	0.0001±0.0000
Muscles	0.0009±0.0003
Ovaries	0.0005±0.0004
Total body	0.0027±0.0010
Effective dose (mSv/MBq)	0.0024±0.0003

Nesidioblastosis is another clinical challenge. The pathogenesis of this disease still remains unclear. An increased expression of insulin-like growth factor 2 receptor (IGF2R), insulin-like growth factor 1 receptor alpha (IGF1Rα) and transforming growth factor beta receptor type 3 (TGFβR3) was observed in samples containing pancreatic islets from patients suffering from nesidioblastosis. It seems that certain growth factors and growth factor receptors may significantly contribute to the development of nesidioblastosis [22]. Reubi et al. evaluated in vitro the GLP-1 receptor status of pancreatic tissues collected from patients suffering from hyperinsulinaemic hypoglycaemia, specifically after gastric bypass surgery. In this study the overexpression of the GLP-1 receptor was not found on the pancreatic islet cell surface. Thus, according to the authors, patients with post-gastric bypass hyperinsulinaemic hypoglycaemia should not be examined with GLP-1 receptor imaging in vivo [23]. However, in clinical practice it should be expected that insulinoma may coexist with nesidioblastosis. Recurrent hypoglycaemias even after surgical resection of 70 % of the pancreas in patients with nesidioblastosis may suggest the presence of insulinoma [24]. In the patient with nesidioblastosis examined with labelled GLP-1 receptor agonist in our department, accumulation of the tracer was observed in the pancreatic area. In this patient histopathological examination confirmed coexistence of insulinoma and nesidioblastosis. Moreover, in one patient suspected of having insulinoma (38-year-old woman), histopathological examination also revealed a microadenoma of the pancreatic head (in the localization of the focal uptake of GLP-1) and coexisting nesidioblastosis. These findings support the statement that, despite similar symptoms resulting from hyperinsulinaemia and hypoglycaemia, the pathogenesis of nesidioblastosis may vary significantly depending on the patient's clinical history and may cause lots of problems for clinicians. The other conclusion is that because of difficulties in the management of patients with nesidioblastosis and the potential coexistence of insulinoma and nesidioblastosis, physicians should include GLP-1 receptor imaging as a diagnostic possibility for these patients.

It should be highlighted that the use of image fusion was mandatory for proper diagnosis in all cases. In some cases the fusion of GLP-1 receptor scintigraphy and CT/MRI enables visualization of small lesions not reported in CT/MRI before. In our group of patients in two cases a focal lesion was found on repeat CT/MRI scans following a positive result of GLP-1 receptor scintigraphy.

Despite the fact that a high kidney uptake was visible in all cases, the image quality was excellent and tumour foci were very well visible with a high T/nT ratio. Background uptake over the whole body was low with the exception of the kidneys, which were strongly labelled owing to renal excretion of the compound. The same finding was reported for ^{111}In-DOTA-exendin-4. Therefore, as it was suggested for GLP-1 receptor imaging with ^{111}In, additional delayed images should be performed in patients with negative early scans [10]. For optimizing the acquisition protocol, Gelofusine, fragmented albumin or other substances should be used for blocking tracer uptake by proximal kidney tubules [11, 25].

Recently, PET imaging radiotracers labelled with ^{68}Ga and ^{18}F, targeting GLP-1 receptors, have also been developed {[Lys^{40}(Ahx-DOTA-^{68}Ga)NH$_2$]exendin-4, [Lys^{40}(^{68}Ga-DOTA)]exendin-3 and ^{18}F-FBEM-EM3106B} [11, 26, 27]. The potential of novel biomarkers based on GLP-1 analogues suitable for PET examinations has been tested only in animal models until now. Because of different advantages and disadvantages of all GLP-1 targeting tracers (labelled with ^{99m}Tc, ^{111}In, ^{68}Ga or ^{18}F) today it is a matter of argument which of them will be the best for detecting insulinomas in humans. Unquestionably, the GLP-1 receptor tracer labelled with ^{99m}Tc is characterized by the general availability of the isotope, lower radiation exposure to patients and staff, and potential usefulness of this compound for intraoperative localization of insulinoma foci using a gamma probe [11]. It is possible that all of them (no matter which radionuclide the GLP-1 analogue is conjugated with) will present the same very high sensitivity and specificity related to an extremely high density of GLP-1 receptors on benign insulinoma cell surface. Nowadays there is a lack of comparative studies with the use of different GLP-1 radiopharmaceuticals injected into the same patients. In the near future such examinations (based on exendin-4 labelled with ^{99m}Tc, ^{68}Ga and ^{111}In) are planned. However, taking into account the very high sensitivity of this method the registration by national authorities of the labelled compound based on GLP-1 analogues might be considered in cases of patients with suspected benign insulinoma in the nearest future. Moreover, it is well known that there are other tumours overexpressing GLP-1 receptors; therefore, the next natural step should be the use of labelled GLP-1 analogues in cases of tumours other than benign insulinoma. In the clinical trial conducted in our department this tracer was used successfully for visualization of medullary thyroid cancer and phaeochromocytoma (unpublished data).

In this paper the biokinetics and initial dose assessment of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 for SPECT/CT studies were also addressed. The average effective dose is comparable with the radiation dose to patients after SRS performed with ^{99m}Tc-EDDA/HYNIC-Tyr^3-octreotide [28, 29]. Wild et al. evaluated the pharmacokinetics and radiation dose to patients after injection of [Lys^{40}(Ahx-HYNIC-^{99m}Tc/EDDA)NH$_2$]-exendin-4 in an animal study [11]. Comparing the biokinetics, our study showed a faster retention of the tracer in human organs assumed to be radiation sources. For organ radiation doses and the effective dose, similar levels of these values were calculated, except for the kidneys (an about twofold higher absorbed dose was calculated in our study). These data should be considered only as the basis for further evaluation of radiation safety of this radiopharmaceutical.

Conclusion

GLP-1 receptor scintigraphy is a promising diagnostic tool for patients with clinical symptoms of insulinoma. It enables the localization of even very small tumours with excellent sensitivity and specificity, and proper imaging is the most important step for successful surgery and complete patient recovery. Despite some difficulties to overcome, the new compound seems to be an effective new tracer for clinical practice and appears to be safe for the patient.

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