Sulfonylurea receptor as a target for molecular imaging of pancreas beta cells with [99mTc]-DTPA-glipizide

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Abstract

Objective—This study was aimed to assess pancreas beta cell activity using [99mTc]-diethyleneaminepentaacetic acid-glipizide (DTPA-GLP), a sulfonylurea receptor agent. The effect of DTPA-GLP on the blood glucose level in rats was also evaluated.

Methods—DTPA dianhydride was conjugated with GLP in the presence of sodium amide, yielding 60%. Biodistribution and planar images were obtained at 30–120 min after injection of [99mTc]-DTPA-GLP (1 mg/rat, 0.74 and 11.1 MBq per rat, respectively) in normal female

Fischer 344 rats. The control group was given ^{99m}Tc-DTPA. To demonstrate pancreas beta cell uptake of ^{99m}Tc-DTPA-GLP via a receptor-mediated process, a group of rats was pretreated with streptozotocin (a beta cell toxin, 55 mg/kg, i.v.) and the images were acquired at immediately—65 min on day 5 post-treatment. The effect on the glucose levels after a single administration (ip) of DTPA-GLP was compared to glipizide (GLP) for up to 6 h.

Results—The structure of DTPA-GLP was confirmed by NMR, mass spectrometry and HPLC. Radiochemical purity assessed by ITLC was >96%. ^{99m}Tc-DTPA-GLP showed increased pancreas-to-muscle ratios, whereas ^{99m}Tc-DTPA showed decreased ratios at various time points. Pancreas could be visualized with ^{99m}Tc-DTPA-GLP in normal rat, however, ^{99m}Tc-DTPA has poor uptake suggesting the specificity of ^{99m}Tc-DTPA-GLP. Pancreas beta cell uptake could be blocked by pre-treatment with streptozotocin. DTPA-GLP showed an equal or better response in lowering the glucose levels compared to the existing GLP drug.

Conclusions—It is feasible to use ^{99m}Tc-DTPA-GLP to assess pancreas beta cell receptor recognition. ^{99m}Tc-DTPA-GLP may be helpful in evaluating patients with diabetes, pancreatitis and pancreatic tumors.

Keywords

^{99m}Tc-DTPA-glipizide; Sulfonylurea receptor; Imaging; Pancreas

Introduction

Diabetes mellitus results from an absolute or relative reduction in pancreatic beta cell mass leading to insufficient secretion and hypoglycemia. Both type 1 and type 2 diabetes are characterized by deficits in beta cell mass: around 99% deficit in long-standing type 1 diabetes [1, 2], and 65% deficit in long-standing type 2 diabetes [3]. Type 1 diabetes results from a selective destruction of the beta cells by a T-cell mediated autoimmune process [4]. Type 2 diabetes begins with insulin resistance in the peripheral tissues, a gradual increase in circulating blood glucose that triggers beta cells to release even more insulin, and subsequent failure of beta cells to maintain normal glucose homeostasis [5]. Recent study suggested that the frequency of beta cell apoptosis is also significantly increased type 2 diabetes [3]. Treatments for diabetes mellitus range from drugs that increase insulin secretion and sensitivity, to insulin administration, to pancreatic islet cell transplantation [6]. Because of the anatomical inaccessibility of the human pancreas for biopsy, noninvasive procedures are needed to quantify beta cell mass in vivo for monitoring the onset, progression and responses to therapy of type 1 and 2 diabetes, and to detect rejection in pancreatic islet transplant patients. Two different approaches have been used to determine the functional beta cell mass in living humans. Information of beta cell mass currently can be deduced from functional tests of insulin secretion, but it has not been shown to correlate well with the dysfunction of beta cells that result from morphological and biochemical changes in the pancreas [3, 7].

During regenerative processes following experimental reduction of beta cell mass such as partial pancreatectomy and streptozotocin injection, beta cell mass increase is not associated with a corresponding improvement of beta cell function, thus indicating that regenerative beta cells did not achieve functional maturity [8]. These studies indicated that anatomical beta cell mass could not always predict functional capacity of the beta cell tissue. Thus, the non-invasive imaging techniques such as MRI, PET and SPECT could be useful to quantify the pancreatic beta cell mass [9].

Insulin secretion is regulated by the membrane potential of the beta cell, which depends on the activity of ATP-sensitive K^+ channels (K_{ATP} channels) in the plasma membrane. The

K_{ATP} channel is a hetero-octameric complex of two different types of protein subunits: an inwardly rectifying K^+ Channel (Kir6.x) and a sulfonylurea receptor. More than one isoform exists for both Kir6.x (Kir6.1, Kir6.2) and sulfonylurea receptors [10]. Sulfonylurea receptor agents stimulate insulin release from pancreatic beta cells, which improves overall glycemia control in patients with type 2 diabetes [11]. While sulfonylurea receptor-1 is expressed in a very high density of at the internal face of the plasma membrane of the pancreatic islet cells but not in the exocrine cells, the sulfonylurea receptor-2 is predominant in the cardiac, skeletal, and smooth muscles cells [11, 12]. Tolbutamide, chlorprop-amide, gliclazide, and glipizide are specific for the pancreatic β-cells sulfonylurea receptor-1, and thus their effect is largely on potentiating insulin secretion [13]. Therefore, these sulfonylurea receptor agents labeled with radionuclide could be a feasible tracer for visualizing the beta cell mass in humans by accumulation at the internal face of the plasma membrane of the pancreatic islet cells by binding to sulfonylurea receptor-1. Recently, glibenclamide, a sulfonylurea receptor agent, was used as a compound candidate to image the pancreatic beta cell in humans. However, they could not obtain a reliable assessment and visualization of the pancreatic islet cell mass due to overall radiochemical yields, low binding affinity, and longer deposition time at the receptor [14, 15]. Schneider et al. suggested that [99mTc]-labeled glibenclamide derivative with a lower lipophilicity and different in vivo behavior may be possible as compound for noninvasive imaging of the pancreatic islet cell mass [16].

Glipizide (GLP) is a potent sulfonylurea receptor agent and is a second generation oral hypoglycaemic agent similar in potency to glibenclamide [17–19]. The efficacy, safety, and dose–response characteristics of glipizide using the gastrointestinal therapeutic system on plasma glucose, glycosylated hemoglobin (HbA_1c), and insulin secretion to a liquid-mixed meal in diabetic patients were evaluated [18]. In this study, glipizide is effective in improving glycemic indices by numerous methods including HbA_{1c}, meal tolerance, and intravenous glucose tolerance testing and there are no significant adverse effects with therapy except for mild to moderate hypoglycemia.

Due to favorable physical characteristics and its low cost, [99mTc] has been preferred for labeling radiopharmaceuticals. Diethylenetriaminepentaacetic acid (DTPA) can be labeled with [99mTc] easily and efficiently with high radiochemical purity and stability and is excreted through kidney [20]. Thus, we developed a noninvasive method to image the beta cell mass by labeling a sulfonylurea receptor agent, GLP with [99mTc] using DTPA as a chelator. In this paper, we report the synthesis, biodistribution and imaging study in normal rats using [99mTc]-DTPA-glipizide (DTPA-GLP). The effect of DTPA-GLP on the blood glucose level in rats was also evaluated.

Materials and methods

Chemicals and analysis

Mass spectral analyses were conducted at The University of Texas Health Science Center (Houston, TX, USA). The mass data were obtained by fast atom bombardment on a Kratos MS 50 instrument (Kratos Instrument, Ltd., Manchester, UK). ^{1}H- and ^{13}C-NMR studies were performed on Bruker 300 MHz spectrometer at the NMR core facility at MD Anderson Cancer Center (Houston, TX, USA). Glipizide is commercially obtained from Acros Organics (New Jersey, USA). All other chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Silica gel-coated thin-layer chromatography (TLC) plates were purchased from Whatman (Clifton, NJ, USA).

Synthesis of DTPA-GLP

The synthetic scheme of DTPA-GLP is shown in Fig. 1. 85.8 mg (2.2 mol) of $NaNH_2$ was added to the solution of 445.5 mg (1.0 mol) of glipizide in 5 mL of dried DMSO. The apparatus was attached with a nitrogen balloon. After stirring for 30 min, 357.3 mg (1.0 mol) of diethylenetriaminepentaacetic acid (DTPA)-dianhydride was placed into the reaction mixture. After stirring over-night at room temperature, 160.5 mg (3.0 mol) of NH_4Cl and 54 µL (3.0 mol) of water was added to the mixture. After stirring for 1 h, the mixture of 1.5 mL of 2 N Na_2CO_3 and 20 mL of water was slowly added into the reaction mixture inside of an ice bath. The pH of the solution was around 7.6 upon removal. This solution was then dialyzed using a membrane with the cut off at MW <1,000 for 5 h. The remaining solution was dried using a lyophilizer. The crude product was isolated through a column packed with Sephadex G-50 and eluted with water. The yield for desired product was 507 mg (61.7%). The structure of DTPA-GLP was confirmed by ^1H- and ^{13}C-NMR and mass spectrometry. High-performance liquid chromatography (HPLC), equipped with a UV detector (220 nm), is performed on a C-18 reverse phase column (Biosep SEC-S3000, 7.8×300 mm, Phenomenex, Torrance CA) using a flow rate of 0.5 mL/min. The eluant was 5 mM ammonium phosphate/acetonitrile (50/50, pH = 8.1).

Radiosynthesis of ^{99m}Tc-DTPA-GLP conjugate (^{99m}Tc-DTPA-GLP)

Radiosynthesis of ^{99m}Tc-DTPA-GLP was achieved through dissolving DTPA-GLP (0.1 mg) in water (0.2 mL), followed by adding tin (II) chloride (0.1 mg, 0.1 mL). Sodium pertechnetate ($NaTcO_4$) (37 MBq) was then added. The final concentration was 370 MBq/µmol. Radiochemical purity was assessed by using a radio-TLC scanner (Bio-scan, Inc., Washington, DC) using saline or acetone as an eluant. The chromatographic paper used was Whatman #1 (Fischer Scientific, Houston, TX).

Biodistribution studies

The animals were housed in The University of Texas M.D. Anderson Cancer Center facility. All protocols involving animals were approved by the University of Texas M.D. Anderson Institutional Animal Use and Care Committee (IACUC). Fischer 344 rats (150 ± 30 g) (Harlan Sprague–Dawley, Indianapolis, IN, USA) ($n = 18$) were injected intravenously with 740 ± 19 KBq of ^{99m}Tc-DTPA-GLP or ^{99m}Tc-DTPA. The injected mass of ^{99m}Tc-DTPA-GLP was 0.1 mg/rat. Separate biodistribution studies using ^{99m}Tc-DTPA-GLP (Study 1, nine rats) and ^{99m}Tc-DTPA (Study 2, nine rats) were conducted. For each compound, the rats were divided into three groups for three time intervals (30, 60 and 180 min, $n = 3$/time point). At 30, 60 and 180 min following administration of the radiotracers, the rats were killed by cervical dislocation. Samples of the blood and selected tissues were obtained, weighed, and subjected to counting along with a standard of the injected dose in a gamma counter (Packard Cobra Auto-Gamma Counter, Packard Instruments, Downers Grove, IL, USA). The bio-distribution of tracer in each sample was calculated as a percentage of the injected dose per gram of tissue wet weight (%ID/g). Pancreas to non-target tissue count density ratios were calculated from the corresponding %ID/g values.

Planar scintigraphic imaging studies

Scintigraphic images were obtained using an M-camera from Siemens Medical Systems (Hoffman Estates, IL, USA). The camera was equipped with a low-energy parallel-hole collimator. The field of view was 53.3×38.7 cm. The intrinsic spatial resolution was 3.2 mm. The matrix is 64×64 and the pixel size was 19.18 mm (32×32, zoom = 1) to 0.187 mm ($1,024 \times 1,024$, zoom = 3.2). With a low-energy, high-resolution collimator, the system has a resultant sensitivity of 172 counts/min (cpm)/µCi and spatial resolution of 4 mm. Scintigraphic images were obtained at 45 min after intravenous injection of 300 µCi (11.1

MBq/rat) of ^{99m}Tc-DTPA-GLP and ^{99m}Tc-DTPA as control in normal healthy female Fischer 344 rats (150–175 g) (Harlan Sprague–Dawley, Inc., Indianapolis, IN, USA) via the tail vein, respectively. Scintigraphic images were acquired 1×10^6 counts. To ascertain whether the pancreas beta cell uptake with ^{99m}Tc-DTPA-GLP was related to sulfonylurea receptors, we performed a blocking study. A group of rats were pretreated with streptozotocin (a beta cell toxin, 55 mg/kg, i.v.) and the images were acquired at 5, 25, and 45 min after intravenous injection of 11.1 MBq/rat of ^{99m}Tc-DTPA-GLP on day 5 post-treatment.

Pharmacological effects of DTPA-GLP on the blood glucose level

To demonstrate the effectiveness of DTPA-GLP in lowering the blood glucose level in Fischer 344 rats, 4 groups of Fischer 344 rats received 300 or 760 mg GLP/kg, 300 or 760 mg DTPA-GLP/kg in single treatment (ip). GLP was dissolved in the cotton seed oil, whereas DTPA-GLP was dissolved in saline. The concentration prepared for both agents was 100 mg/mL in animal studies. The blood glucose levels were measured with the tail-bleeding technique [21] using a blood glucose meter (Bayer's Breeze®, Bayer HealthCare, NY, USA) immediate before injection, 5 min to 60 min with an interval of 5, 90, 120, 180, 240, and 360 min post-injection.

Statistical analysis

The in vivo percentage of injected dose per gram of wet tissue weight (%ID/g), pancreas-to-normal muscle and pancreas-to-blood count ratios were presented as means ± standard errors of the means. To compare differences of %ID/g and ratios between control and DTPA-GLP groups, the Student t test was used. All analyses were 2-tailed and considered as type 2. A p value <0.05 was considered statistically significant. All statistical computations were processed by using a computer software program (Excel, Microsoft, Redmond, WA, USA).

Results

Chemistry and radiochemistry

HPLC analysis showed the retention time of DTPA-GLP was 5.738 min with purity greater than 93%. ^{1}H-NMR data of DTPA-GLP is ^{1}H-NMR (300 MHz, D$_2$O) δ ppm 8.65 (s, 1H), 8.28 (s, 1H), 7.50 (d, 2H, $J = 8.2$ Hz), 7.17 (d, 2H, $J = 8.2$ Hz), 3.34 (s, 2H), 3.12 (s, 4H), 3.02 (s, 4H), 2.89 (t, 2H, $J = 6.5$ Hz), 2.75–2.83 (m, 1H), 2.71 (t, 2H, $J = 6.5$ Hz), 2.51 (s, 8H), 2.36 (s, 3H), 1.30–1.40 (m, 4H), 0.77–1.01 (m, 6H) (Fig. 2). ^{13}C-NMR (75 MHz, D$_2$O) δ ppm 179.5, 177.9, 165.8, 161.7, 157.9, 144.6, 143.5, 142.2, 142.0, 141.5, 129.6, 126.7, 59.1, 58.6, 56.4, 52.9, 51.4, 49.4, 40.7, 39.2, 34.9, 33.1, 25.5, 24.8, 21.0 (Fig. 3). The molecular weight of DTPA-GLP is 820.87. The mass spectrum of DTPA-GLP was confirmed by formylated DTPA-GLP (Fig. 4). The radiochemical purity of ^{99m}Tc-DTPA-GLP was 97.2% (eluted with saline or acetone) determined by radio-instant thin-layer chromatogram.

Biodistribution studies

The biodistribution of ^{99m}Tc-DTPA and ^{99m}Tc-DTPA-GLP in normal Fischer 344 rats is shown in the Tables 1 and 2, respectively. The uptake of ^{99m}Tc-DTPA-GLP in the kidneys was high, indicating that the radiotracer was excreted mainly through the urine. Pancreatic tissue displayed a stable accumulation of ≈0.25 and 0.21% of the injected ^{99m}Tc-DTPA-GLP dose at 30 and 60 min, respectively. Pancreas uptake (% ID/g) in ^{99m}Tc-DTPA-GLP was significantly higher than ^{99m}Tc-DTPA at 1 and 3 h (0.21 ± 0.00 vs. 0.08 ± 0.01, 0.15 ± 0.01 vs. 0.05 ± 0.01, p <0.05, respectively). Pancreas-to-muscle ratios were higher in ^{99m}Tc-

DTPA-GLP group than in ^{99m}Tc-DTPA groups. At 2 h, pancreas-to-muscle ratio ^{99m}Tc-DTPA-GLP was significantly higher than ^{99m}Tc-DTPA (2.76 ± 0.07 vs. 1.74 ± 0.23, p <0.05). Generally, blood, heart, and lung uptakes in ^{99m}Tc-DTPA-GLP groups were higher than ^{99m}Tc-DTPA groups. In contrast, tracer uptake in the kidney was lower in ^{99m}Tc-DTPA-GLP group than in ^{99m}Tc-DTPA group. There was virtually no retention of radioactivity in the brain during the observation period.

Scintigraphic imaging

Planar images of the rats injected with two different compounds, ^{99m}Tc-DTPA and ^{9m}Tc-DTPA-GLP are shown in Fig. 3. Because thyroid and stomach activities were not detected on the planar images, it was considered to signify the in vivo stability of the radiopharmaceuticals. The pancreas could be visualized with ^{99m}Tc-DTPA-GLP in normal rats; however, ^{99m}Tc-DTPA has no definite, pancreas uptake suggesting the specificity of ^{99m}Tc-DTPA-GLP (Fig. 5). The surrounding organs (liver, spleen and intestine) around the pancreas showed faint uptakes of ^{99m}Tc-DTPA-GLP. In contrast, because DTPA was used as chelator, radioactivies in both the kidneys and urinary bladder were higher compared to the pancreatic uptake. In blocking studies with streptozotocin, pancreas beta cell uptake could be blocked by pre-treatment with streptozotocin; therefore, the pancreas could not be visualized in rats pretreated with streptozotocin in comparison with the untreated rat (Fig. 6). The finding indicated that pancreas beta cell uptake of ^{99m}Tc-DTPA-GLP is via a sulfonylurea receptor-mediated process.

Pharmacological effects of DTPA-GLP on the blood glucose level

Figure 7 illustrates the effect of GLP and DTPA-GLP on the blood glucose levels in Fischer 344 rats. Rats died at 760 mg GLP/kg within 40 min after injection suggesting maximum tolerated dose achieved, whereas rats survived at 760 mg DTPA-GLP/kg in single treatment. The blood glucose levels were decreased by GLP and DTPA-GLP at the dosage tested. DTPA-GLP had faster onset and was more steady than GLP.

Discussion

In this paper, ^{99m}Tc-DTPA-GLP was synthesized for a novel agent to visualize the pancreatic beta cell mass. ^{99m}Tc-DTPA-GLP accumulated specifically in the pancreatic islet cell in the rats. To our knowledge, glipizide was labeled for the first time with ^{99m}Tc. We labeled SUR agent, GLP with ^{99m}Tc using DTPA as a chelator. Radio-TLC results with ^{99m}Tc-DTPA-GLP confirm the high radiochemical purity and stability. Due to favorable physical characteristics and its low cost, ^{99m}Tc has been chosen as a radionuclide instead of positron emitters (^{18}F or ^{11}C) used in other studies [14, 15, 22, 23]. Because DTPA can be labeled with ^{99m}Tc easily and efficiently with high radiochemical purity and stability and is also excreted through the kidney [20], DTPA was selected to synthesize a new ^{99m}Tc-DTPA-GLP as a chelator. Several compounds have been labeled with ^{99m}Tc using DTPA as a chelator [24–26].

Our biodistribution studies with Fischer 344 rat administrated ^{99m}Tc-DTPA-GLP revealed that a large portion of the injected dose (%ID/g) was rapidly accumulated by the kidneys, and pancreatic tissue displayed a stable accumulation of ≈ 0.25 and 0.21% of the injected ^{99m}Tc-DTPA-GLP dose at 30 and 60 min, respectively. Direct comparison of the biodistribution of GLP is difficult because there are no reports on the beta cell imaging with GLP labeled with gamma or positron emitters. There are two studies with ^{18}F-glyburide [14], and 2-[^{18}F]fluoroethoxy-5-bromogli-bendamide and ^{99m}Tc-glibenclamide, as the second generation sulfonylurea like GLP [14].

Tissue distribution of 2-[^{18}F]fluoroethoxy-5-bromogli-benclamide in rats showed that pancreatic tissue displayed a weak but near constant accumulation of the tracer with 0.084 ± 0.01 %ID/g, and the tracer accumulation in the liver was approximately 100-fold higher compared to the pancreas uptake. In contrast, the tissue distribution of ^{99m}Tc-glibenclamide showed that the pancreas uptake was only reduced (tenfold) when compared with 2-[^{18}F]fluoroethoxy-5-bromoglibenclamide at 1 h after tracer application. Schmitz et al. [15] reported similar results of tissue distribution with ^{18}F-glyburide similar to previously mentioned study with 2-[^{18}F]fluoroethoxy-5-bromoglibenclamide and ^{99m}Tc-glibenclamide. Our data showed that tracer accumulation in the pancreas was approximately 30-fold higher compared to that of these data described above.

Unlike studies with ^{18}F-glyburide [15], and 2-[^{18}F]fluoroethoxy-5-bromoglibendamide and ^{99m}Tc-glibenclamide [16], we found lower ^{99m}Tc-DTPA-GLP uptake in the organs (the liver and intestine) around the pancreas, which is an another advantage of ^{99m}Tc-DTPA-GLP because of the proximity of these organs to the pancreas.

In our study, biodistribution findings were comparable to scintigraphic imaging (Figs. 5, 6). We did not find thyroid and stomach activities on the planar images, strongly indicating the in vivo stability of the radiopharmaceuticals. The pancreas could be visualized with ^{99m}Tc-DTPA-GLP in normal rats. Because we selected DTPA as chelator to accelerate urinary excretion of the tracer, radioactivities in both the kidneys and urinary bladder were higher compared to the pancreatic uptake. The optimal time to image pancreas of ^{99m}Tc-DTPA-GLP was at 45–65 min. Heart and muscle had poor uptake at 45–65 min suggesting ^{99m}Tc-DTPA-GLP is for the pancreatic β-cells sulfonylurea receptor-1.

Streptozotocin is widely used to induce diabetic animal model [15, 27, 28]. Administration of a beta cell-specific toxin streptozotocin could induce diabetes in the F-344 rats and we have used the same animal model in our studies. The dosage to create the animal model is known [28]. Also, streptozotocin is a therapeutic agent in the treatment of patients with pancreatic neuroendocrine tumors. Streptozotocin destroyed beta cells to avoid pancreatic neuroendocrine tumor adherence [29]. Thus, streptozotocin was selected instead of sulfonylurea receptor agents in our blocking studies.

Streptozotocin enters the beta cell via a glucose transporter-2 and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, which leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, beta cells undergo the destruction by necrosis [30]. In this study, scintigraphic imaging of the streptozotocin-treated rat showed no uptake of ^{99m}Tc-DTPA-GLP in the pancreas compared to baseline studies. The finding indicated that pancreas beta cell uptake of ^{99m}Tc-DTPA-GLP is via a sulfonylurea receptor-mediated process.

Our study showed that DTPA-GLP have a similar ability as GLP itself to decrease the blood glucose levels. This indicates that DTPA-GLP binds to sulfonylurea receptors and then stimulates the insulin secretion from pancreatic beta cells in rat. The physical amount of DTPA-GLP will be 3–10 mg/70 kg. Pharmacological effect of glipizide is at the dosage level of 300 mg/kg (i.v.). Thus, the proposed dosage is 2,100–7,000 times less than pharmacological dose.

In conclusion, DTPA-GLP was synthesized and labeled with ^{99m}Tc easily and efficiently, with high radiochemical purity and cost-effectiveness. We found that the use of SPECT

and ^{99m}Tc-DTPA-GLP provides a means to visualize the pancreatic beta cell mass and to discriminate differences in its uptakes among normal healthy rodents and those with streptozotocin-induced diabetes. Therefore, ^{99m}Tc-DTPA-GLP may be helpful to predict and select the patient candidate who may respond to beta cell therapy.

Acknowledgments

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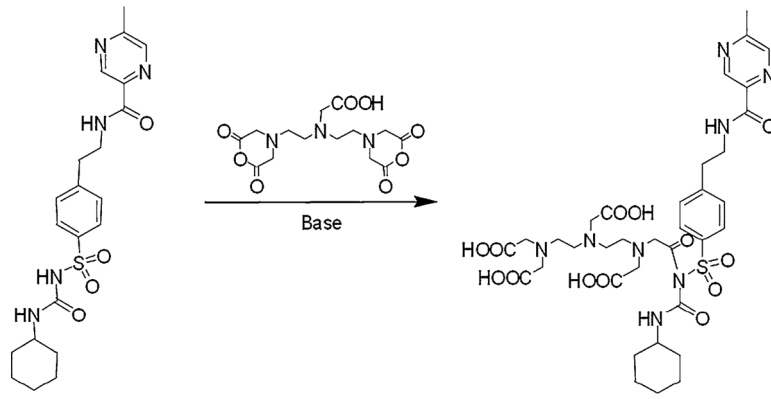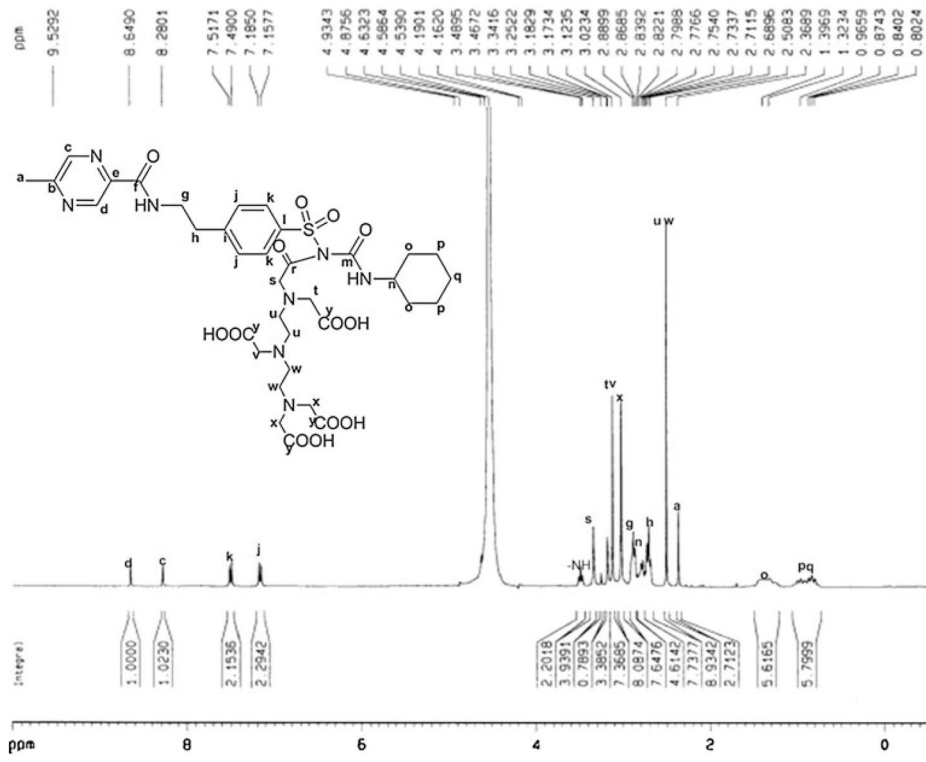
Fig. 1.
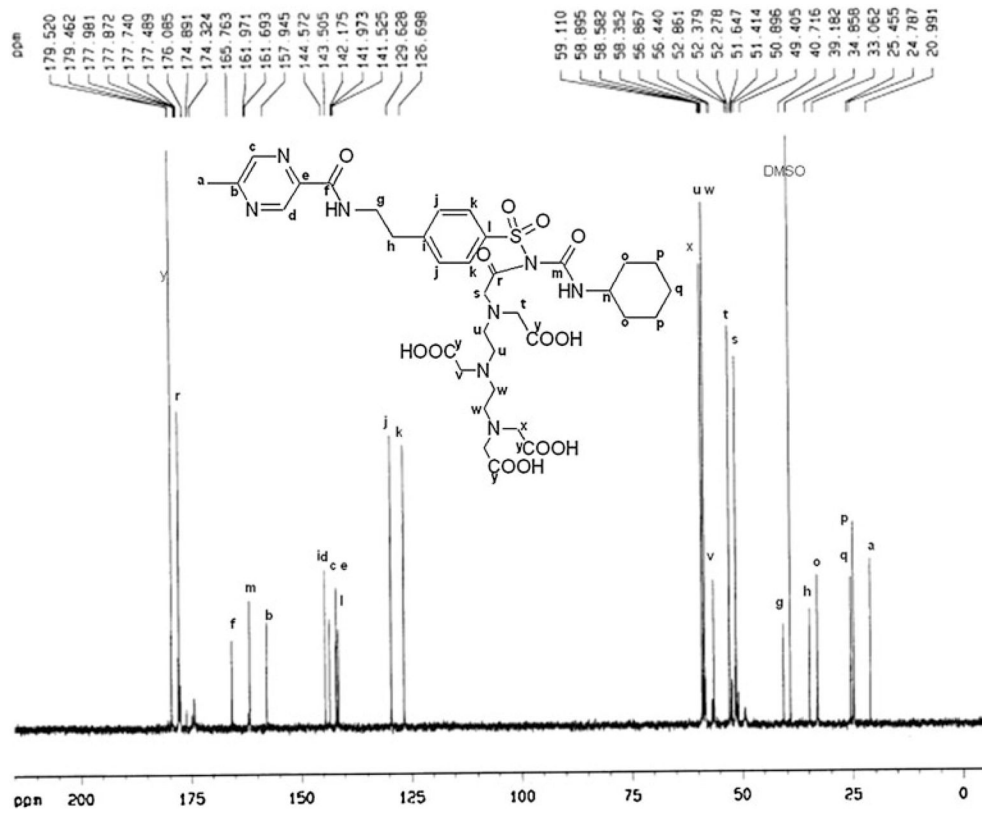Synthetic scheme of DTPA-GLP

Fig. 2.
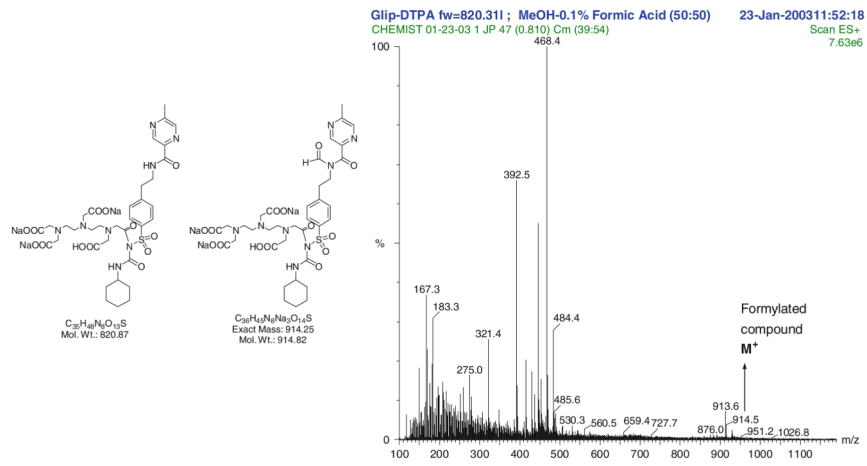^{1}H-NMR spectrum of DTPA-GLP

Fig. 3.
^{13}C-NMR spectrum of DTPA-GLP

Fig. 4.
Mass spectrum of DTPA-GLP

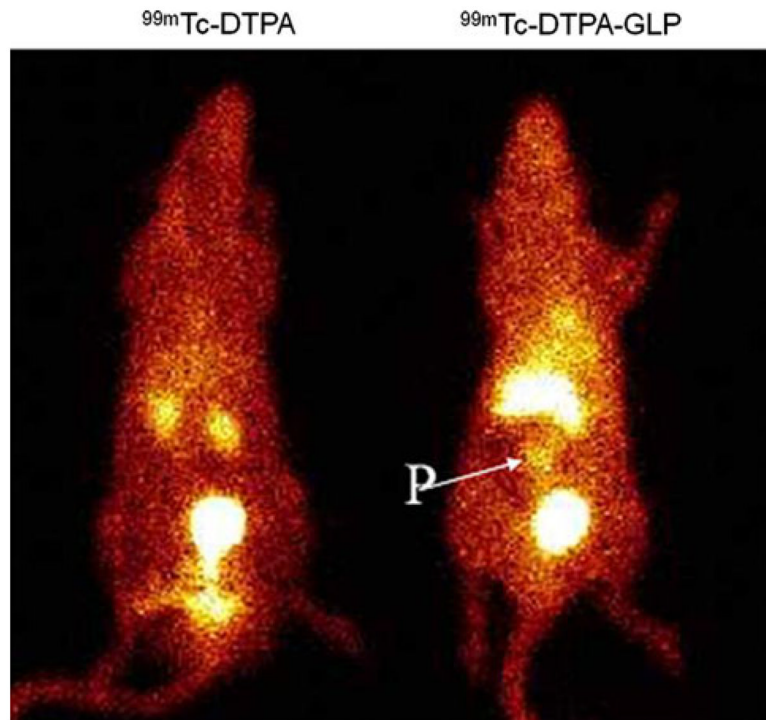**Fig. 5.**
Planar scintigraphy of ^{99m}Tc-DTPA and ^{99m}Tc-DTPA-GLP in rats (11.1 MBq/rat, i.v. injection) at 45 min. Pancreas (*P*) can be visualized by ^{99m}Tc-DTPA-GLP
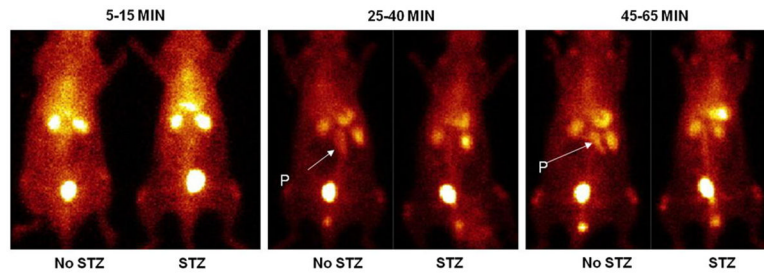
Fig. 6.
Planar scintigraphy image after administration of ^{99m}Tc-DTPA-GLP in rats (11.1 MBq/rat, i.v. injection). The first rat received 55 mg/kg streptozotocin (STZ) 5 days prior to injection. It showed the pancreas could be well visualized 25–65 min post-injection only in the rat which did not receive STZ. Scintigraphic images were acquired 1×10^6 counts, *P* pancreas
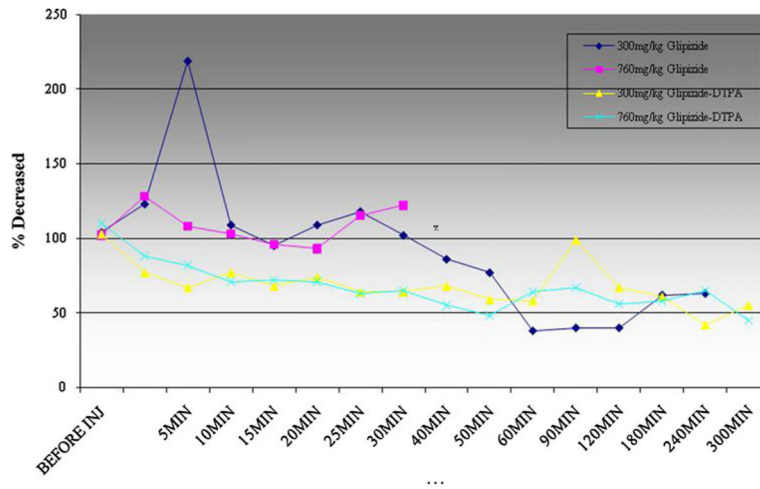
Fig. 7.
Pharmacological Effect of DTPA-GLP on the blood glucose level in Fischer-344 rats. The blood glucose levels were decreased by GLP and DTPA-GLP at the dosage tested. DTPA-GLP had faster onset and more steady than glipizide. Rats died at 760 mg GLP/kg within 40 min after injection suggesting maximum tolerated dose achieved, whereas rats survived at 760 mg DTPA-GLP/kg in single treatment

Table 1

Biodistribution of ^{99m}Tc-DTPA in normal healthy F-344 rats

	30 min	60 min	180 min
Blood	1.08 ± 0.21	0.37 ± 0.04	0.19 ± 0.01
Heart	0.27 ± 0.04	0.10 ± 0.01	0.06 ± 0.01
Lung	0.56 ± 0.08	0.24 ± 0.02	0.14 ± 0.01
Thyroid	0.54 ± 0.18	0.21 ± 0.02	0.19 ± 0.03
Pancreas	0.20 ± 0.00	0.08 ± 0.01	0.05 ± 0.01
Liver	0.80 ± 0.20	0.76 ± 0.04	0.52 ± 0.09
Spleen	0.75 ± 0.11	0.97 ± 0.13	0.76 ± 0.16
Kidney	8.38 ± 1.17	11.33 ± 0.49	10.20 ± 1.17
Stomach	0.39 ± 0.06	0.20 ± 0.05	0.14 ± 0.02
Intestine	0.38 ± 0.00	0.24 ± 0.07	0.12 ± 0.02
Brain	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.12 ± 0.01	0.04 ± 0.01	0.02 ± 0.00
Bone	0.27 ± 0.04	0.15 ± 0.02	0.08 ± 0.01
P/blood	0.19 ± 0.04	0.20 ± 0.01	0.25 ± 0.02
P/muscle	1.72 ± 0.10	1.74 ± 0.23	1.92 ± 0.19

Values are % of injected dose per gram of tissue weight and represent the mean ± standard errors of the mean of data from 3 animals per time interval

P pancreas

Table 2

Biodistribution of ^{99m}Tc-DTPA-glipizide in normal healthy F-344 rats

	30 min	60 min	180 min
Blood	2.08 ± 0.11[*]	1.66 ± 0.04[*]	1.25 ± 0.04[*]
Heart	0.49 ± 0.04[*]	0.33 ± 0.02[*]	0.29 ± 0.03[*]
Lung	0.84 ± 0.01[*]	0.72 ± 0.05[*]	0.52 ± 0.02[*]
Thyroid	0.70 ± 0.14	0.54 ± 0.02[*]	0.41 ± 0.01[*]
Pancreas	0.25 ± 0.03	0.21 ± 0.00[*]	0.15 ± 0.01[*]
Liver	1.10 ± 0.04	1.01 ± 0.05[*]	0.78 ± 0.00[*]
Spleen	0.72 ± 0.03	0.74 ± 0.04	0.60 ± 0.02[*]
Kidney	5.78 ± 0.30	7.52 ± 0.33[*]	7.38 ± 0.29
Stomach	0.25 ± 0.01	0.20 ± 0.00	0.16 ± 0.01
Intestine	0.59 ± 0.11	1.66 ± 0.04	0.25 ± 0.01[*]
Brain	0.06 ± 0.01	0.04 ± 0.01[*]	0.04 ± 0.00[*]
Muscle	0.10 ± 0.00	0.08 ± 0.00[*]	0.06 ± 0.00[*]
Bone	0.20 ± 0.03	0.11 ± 0.01	0.14 ± 0.02[*]
P/blood	0.12 ± 0.01	0.13 ± 0.01[*]	0.12 ± 0.00[*]
P/muscle	2.54 ± 0.26	2.76 ± 0.07[*]	2.50 ± 0.18

Values are % of injected dose per gram of tissue weight and represent the mean ± standard errors of the mean of data from 3 animals per time interval

[*] p <0.05 vs. ^{99m}Tc-DTPA