Preclinical evaluation of gastrin derivatives labelled with ^{111}In: Radiolabelling, affinity profile and pharmacokinetics in rats

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Background. Cholecystokinin receptor subtype 2 (CCK-2) is overexpressed in various tumours like medullary thyroid carcinomas and small cell lung cancer. Radiolabelled peptides that bind with high affinity and specificity to CCK-2 receptors, thus hold great potential for visualizing such tumours.
Methods. We compared four ^{111}In labelled gastrin analogues, called minigastrins (MG), namely MG11, MG45, MG47 and MG48 linked to metal chelating DOTA in preclinical experiments. The radiolabelled peptides were tested for peptide binding in CCK-2 receptor-bearing cell line AR42J and for their pharmacokinetics in normal rats.
Results. The experiments suggest that all gastrin analogues had similar and relatively rapid internalization into AR42J cells. Binding to CCK-2 receptors in AR42J cells was saturable for all agents but there were some differences in receptor affinity. This biodistribution study in rats showed a rapid decrease in blood radioactivity, predominantly renal clearance and saturable uptake of the radiopharmaceutical and/or its metabolites in the CCK-2 receptor-positive stomach. Higher kidney accumulation of radioactivity was only found for ^{111}In-DOTA-minigastrin 48.
Conclusions. The data suggest that the ^{111}In-DOTA-minigastrin analogues studied are promising candidates for the scintigraphy of CCK-2 receptor-expressing tumours; ^{111}In-DOTA-MG47 and ^{111}In-DOTA-MG11 are the most promising.

Key words: peptides, cholecystokinin receptors, receptor targeting, minigastrins, indium-111, radiolabelling, preclinical evaluation

Received: January 15, 2013; Accepted with revision: August 22, 2013; Available online: September 27, 2013
http://dx.doi.org/10.5507/bp.2013.064

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INTRODUCTION

Radiolabelled receptor-specific peptides hold promise for early diagnosis or treatment of diseases. This approach is of particular interest for application in oncology because human cancer cells overexpress many peptide receptors as molecular targets[1,2]. As cholecystokinin receptor subtype 2 (CCK-2) is overexpressed in a high percentage in a large number of neuroendocrine tumours, in particular medullary thyroid carcinoma, small cell lung cancer, astrocytomas, and neuroendocrine gut tumours, CCK-2 receptor which also has high affinity for gastrin, might be a suitable target for radionuclide imaging and therapy[3-6] . For this reason, the development of radiolabelled CCK2-receptor targeting peptides has gained relevant interest in both cancer visualization and receptor-mediated therapy. For routine use in nuclear medicine, it is preferable to employ peptides which are linked to a chelating agent that can be labelled with diagnostic or therapeutic radionuclides. Behr et al.[7] developed DTPA (diethylenetriaminepentaacetic acid)-derivatized *D*-Glu^1-minigastrin (MG0); the peptide labelled with ^{111}In or ^{90}Y had high uptake in stomach and tumour lesions in patients with metastatic thyroid medullary carcinomas[8]. Unfortunately, its uptake in receptor-positive tissues was followed by extremely high radioactivity uptake in the kidney and this limits its application in humans. Kidney uptake of radioactivity is probably related to the penta-Glu motif in position 2-6 in the peptide sequence[9]. Over the past few years, a number of radiolabelled chelator-coupled CCK analogues and gastrin derivatives (called minigastrins) have been developed and tested both in vitro and in vivo[4,5,10,11]. However, a number of issues need to be resolved for clinical application of such receptor-specific peptides. A high receptor affinity to CCK-2 receptors does not necessarily mean more favorable characteristics of the radiolabelled peptide in receptor-mediated radionuclide diagnosis and therapy. For this reason, the optimal structure of these CCK-2 receptor-targeting vectors, combining the high tumour uptake of radioactivity with low kidney retention is, however, still under debate. The aim of this study was to investigate receptor binding affinity and uptake of four ^{111}In-labelled DOTA-minigastrin analogues (DOTA = [1,4,7,10-tetraazacyclo-dodecane-N,N',N'',N'''tetraacetic acid]) in *in vitro* conditions and in preclinical experiments. The agents were tested for their specific binding on the CCK-2 receptor-bearing cell line AR42J derived from the rat exocrine pancreatic tumour. Biodistribution and elimination characteristics of peptides under study in normal rats were also determined. Since CCK-2 receptors are also expressed in normal tissues, the organ bearing a high density of CCK-2 receptors, namely the stomach[12],

served as an endogenous indicator of the specific binding of radiopeptides under study to these receptors in *in vivo* conditions.

MATERIALS AND METHODS

Chemicals

DOTA-minigastrin conjugates used are listed below.
DOTA-MG11:
DOTA-*D*Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
DOTA-MG45:
DOTA-(*D*Gln)$_6$-Ala-Tyr-Gly-Trp-Nle-Asp-Phe-NH_2
DOTA-MG47:
DOTA-(*D*Gln)$_6$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
DOTA-MG48:
DOTA-(*D*Glu)$_6$-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

The peptides were purchased from PiChem, Linz, Austria

They were coupled with bifunctional chelating agent DOTA to hold the ^{111}In with high stability as DOTA forms thermodynamically and kinetically stable complexes with trivalent radiometals.

$^{111}InCl_3$ was obtained from Perkin Elmer,Inc., Boston, MA, USA. All other chemicals were purchased from Sigma-Aldrich and were of an analytical grade.

RPMI 1640 medium and fetal calf serum were purchased from the PAA Cell Culture Company. L-glutamine and ethylenediaminetetraacetic acid (EDTA)/trypsin were purchased from Sigma-Aldrich, Czech Republic. Phosphate buffered saline (PBS) was prepared in our department comprising (mM): NaCl 137, KCl 2.7, Na_2HPO_4 0.01 and NaH_2PO_4 0.01 (titrated to pH 7.4). An acid wash buffer was also prepared in our department in a composition of 50 mM glycine buffer pH 2.8 and 0.1 M NaCl.

Cell line

The rat pancreatic tumor cell line AR42J was purchased from the European Collections of Cell cultures (ECACC).

Radiolabeling with ^{111}In

Radiolabeling was performed by adding about 0.5 mCi $^{111}InCl_3$ in 0.5 - 1 μL of 50 mM HCl to 50 μL 0.4 M sodium acetate buffer pH 4,5 with 10 μg of the peptide. After incubating at 80 °C for 30 min, the quality control of the product was determined by a gradient HPLC analysis. For biological experiments ^{111}In-DOTA-minigastrins were diluted with saline to a concentration of 1 μg/mL.

Determination of Radiochemical Purity

To 30 μL 0.1% TFA (mobile phase A), 10 μL 10^{-3}M DTPA was added together with 2 μL of labeled peptide solution. The HPLC analysis involved a gradient elution performed on the Agilent HPLC System 1100 Series equipped with a LichroCART 250-3 LiChrospher 100 RP-18 (5 μm, Merck) with a UV monitor and a radioactivity monitoring analyzer in 0.1% TFA in water as a mobile phase A and 0.1% TFA in CH_3CN as phase B.

Gradient: 0-10 min 0% B
10-15 min 0-80% B
15-25 min 80-10 % B
25-30 min 100-0% B
The flow rate was 1 mL/min.

Cell culturing

Rat exocrine pancreatic tumour cells AR42J express gastrin/CCK-2, somatostatin and epidermal growth factor (EGF) receptors. AR42J cells were grown in RPMI-1640 (supplemented with 2 mM glutamine and 10% foetal calf serum) in air containing 5% CO_2 at 37 °C. Subculturing was performed employing a trypsin/EDTA solution.

In vitro internalization studies

On the day of the experiment, the cells were treated with a trypsin/EDTA solution and concentrated to 1.10^6 cells per 1 mL of internalization medium (RPMI-1640 supplemented with 2 mM L-glutamine and 1% FCS) per microcentrifuge tube. Incubation was started by adding 10 ng of radiolabelled peptide per tube.
Cells were incubated at 37 °C in triplicate for the indicated time periods. Cellular uptake was stopped by removing the medium and washing the cells with ice-cold PBS (phosphate-buffered saline) twice. The cells were then incubated twice at laboratory temperature in acid wash buffer (50 mM glycine buffer pH 2.8, 0.1 M NaCl) for 5 min. Cells were lysed by treatment in 1M NaOH and cell radioactivity collected (internalized radioligand fraction). Radioactivity was determined using a gamma-counter.

Determination of minigastrin receptor affinity profiles

For each of the tested compounds, complete displacement experiments were done with the unlabelled minigastrin using increasing concentrations. IC_{50} values were calculated after quantifying the data using GraphPad Prism 5 computer-assisted image processing system.

Animals

Animal studies were carried out using male Wistar rats weighing 180-280 g. During the experiment the rats were kept in standard animal facilities which comply with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The animals were fed pelleted food and had free access to both food and water. They were fasted overnight before the experiment (to empty the bowels) but had free access to water. All animal experiments were approved by the Ethics Committee of the Faculty of Pharmacy, Charles University, Hradec Králové.

Biodistribution in rats

The agent was administered to rats intravenously to a volume of 0.2 mL (1 microgram of the peptide per animal). During the course of the experiments, the animals were placed separately in cages. At various points in time after injection, the carotid artery was exposed under ether anesthesia and a blood sample was collected in glass tubes containing dry heparin. The rats were sacrificed and dis-

sected. To determine the effect of the CCK-2 receptor blockade on the peptide distribution profile, per agent one group of animals was pretreated with an intravenous injection of 0.1 mg/kg of the cold (non-radiolabelled) DOTA-minigastrin under study for 15 min before the radiolabelled peptide administration. The organs of interest were weighed and counted for radioactivity in an automatic gamma counter 1480 Wizard 3 (LKB/Wallac).

RESULTS

Radiolabelling with ^{111}In

Labelling of DOTA-minigastrins was achieved by incubating the agents at 80 °C for 15 min in the presence of $^{111}InCl_3$. This procedure resulted in high radiolabelling yields. Radiochemical purity was in the range of 95-99% and no subsequent purification step was used. An example of HPLC analysis of selected radiolabelled peptide has been shown in Fig. 1.

In vitro internalization studies

Rat exocrine pancreatic tumor cell line AR42J expressing gastrin/CCK2 receptor exhibited accumulation of all peptides (Fig. 2). Rapid uptake was observed during the first minutes after administration. The highest uptake was in ^{111}In-DOTA-MG47, the lowest in ^{111}In-DOTA-MG48. The equilibrium of peptide uptake in the cells was not achieved even after 180 min of experiments. A gradual increase of radioactivity uptake in the cells in longer time intervals (after one hour incubation) was recorded.

Receptor affinity studies

Fig. 3 shows the IC_{50} values of minigastrins. The values were obtained by perfoming competitive displacement experiments with unlabelled minigastrin in AR42J cells after 2 h of incubation. Concentrations from 0 to 10 000 nM of unlabelled peptide were monitored.

Biodistribution in rats

After intravenous administration of ^{111}In-DOTA-minigastrins under study to normal rats, radioactivity was rapidly cleared from the blood and other organs for all agents (Table 1 - 4). Specific radioactivity uptake was observed in the receptor-positive stomach. The kidneys (the main elimination organ) were the only non-receptor positive tissue in which high radioactivity accumulation was also observed. In the shortest time intervals, high kidney radioactivity was evidently due to the elimination of radiopeptide into the urine. Elevated and long-term radioactivity localization in the kidney was determined only for ^{111}In-DOTA-MG48. The other organs showed fast clearance with a low background uptake. In other organs and tissues not shown in Tables 1-4, less than 0.2% dose per organ (for the adrenals, pancreas, thyroid and femur) or less than 0.5% dose per gram tissue (for the skin, muscle and fat) were determined. In order to saturate specific binding to CCK-2 receptors, non-radioactive DOTA-minigastrin of the same structure was injected before a selected radiolabelled peptide administration. As predicted, activity in CCK-2 receptor-rich organ (namely in the stomach) at 120 min postinjection was substantially decreased in pre-treated animals (Table 1-4). While blood radioactivity levels were almost identical for control and pre-treated groups, the degree of radioactivity retention in the stomach was substantially lower for CCK-2 receptor-blocked animals. Nevertheless these differences are not statistically significant due to the large range of values in the group of animals without premedication.

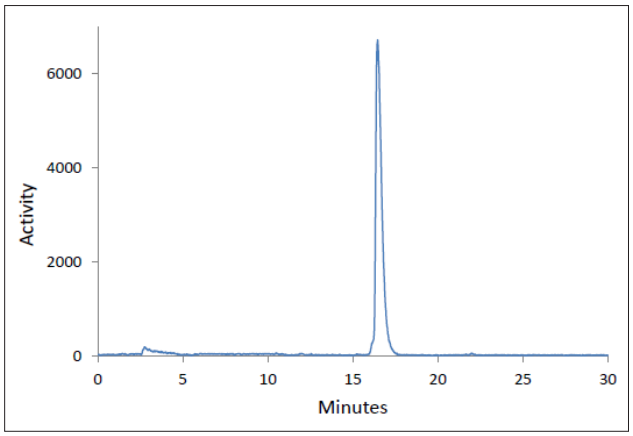

Fig. 1. HPLC analysis of ^{111}In-DOTA-MG-45.

Elimination in rats

The radiolabelled peptides were administered to the rats as described above. The animals were then separately placed into glass metabolic cages, the construction of which allows reliable separation of urine and solid excrements. The animals had free access to standard diet and water. Two hours after administration, the rats were forced to empty their urinary bladders by handling (immobilization) and urine and faeces were collected. The procedure was repeated at 24 h and 48 h intervals after administration.

Fig. 4 are the results of cumulative excretion of radioactivity in the urine and faeces during 48 h after administration. The main elimination pathway was urinary excretion. Most of the radioactivity eliminated through urine was excreted during the first two hours after dosing.

DISCUSSION

Radiolabelled minigastrins represent a new class of peptide vectors with promising results in targeted receptor scintigraphy and radionuclide therapy of some tumours and their metastases[2]. A number of agents have been developed but the optimal structure of the peptide is still under debate. Improvements in pharmacokinetic behavior need to be made, including unfavorable excretion route, rapid metabolism and long-term radioactivity retention in the kidney. In this study, four ^{111}In-DOTA-chelated minigastrins were tested in order to evaluate and compare their preclinical behavior.

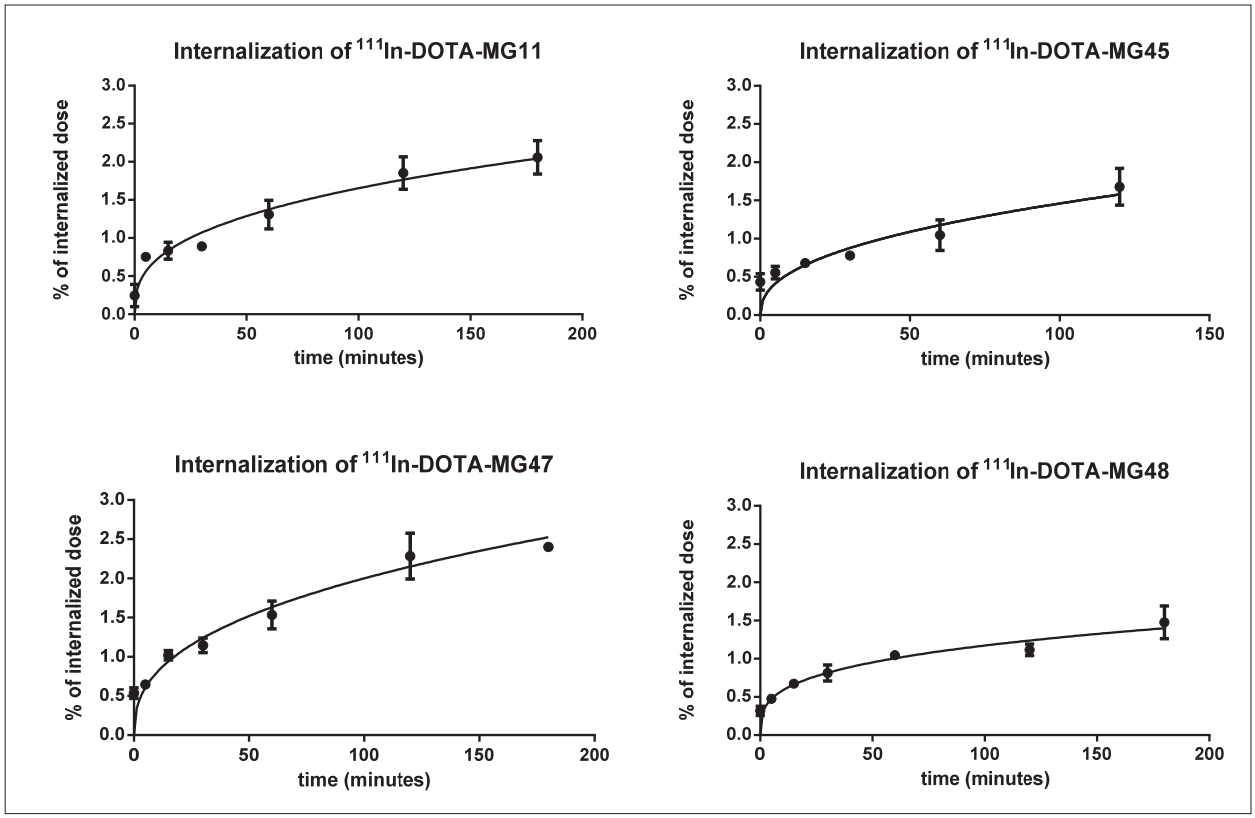

Fig. 2. Internalization of 111In-DOTA-minigastrins in AR42J cells. Data expressed as mean±SD.

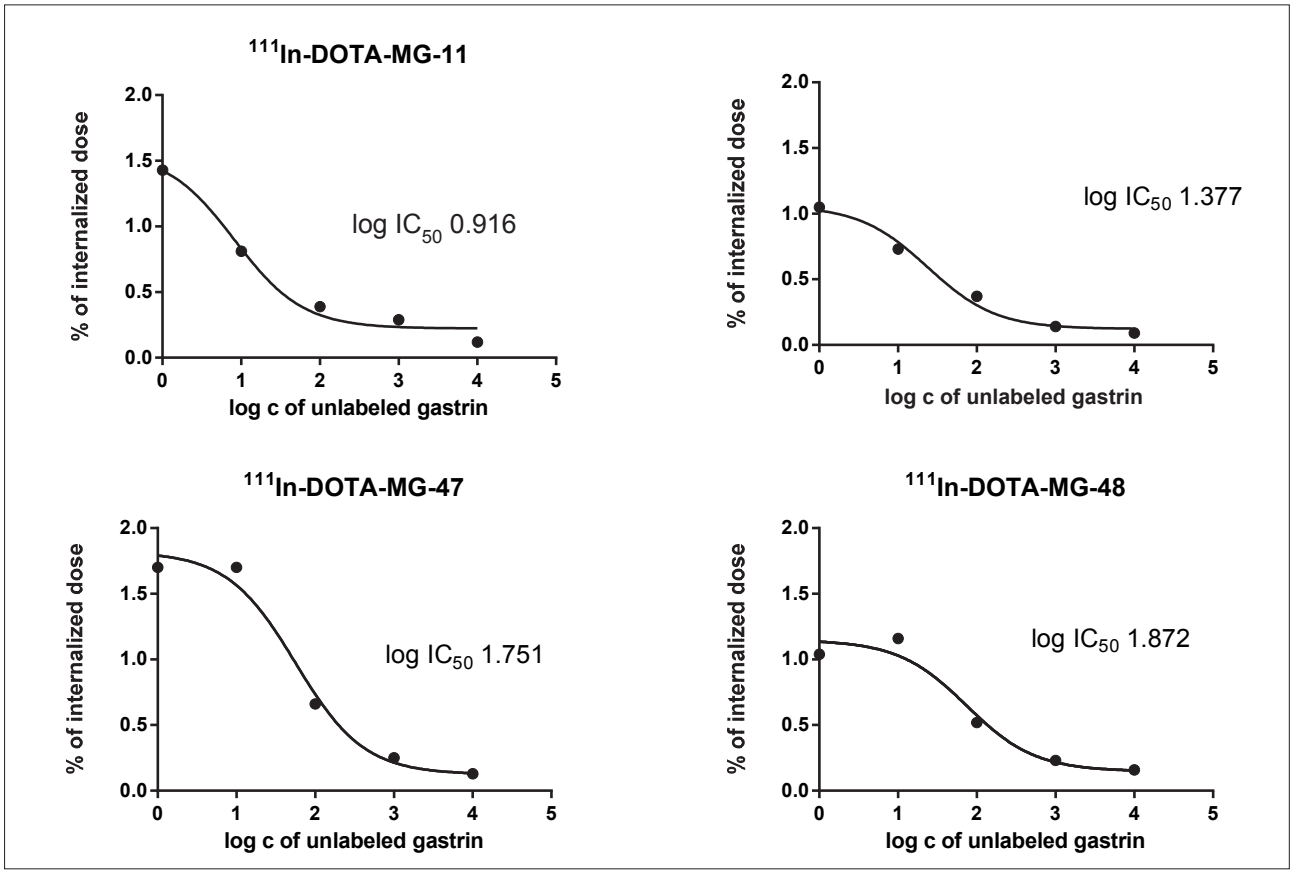

Fig. 3. Receptor affinity studies of 111In-DOTA-minigastrins.

Table 1. Radioactivity uptake in selected organs and tissues of rats after intravenous ^{111}In-DOTA-MG-11 and the effect of CCK-2 receptor blockade (animals were pretreated with the same peptide 0.1 mg/kg of body weight 15 min before the radiolabelled peptide dosing) 120 min after administration. The results are expressed as the percentage of injected dose per gram of the tissue (mean ± SD of four animals).

^{111}In-MG 11 %D/g	5 min	60 min	120 min	120 min premedication	24 h	48 h
Blood	0.94 ± 0.08	0.10 ± 0.02	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Plasma	1.58 ± 0.11	0.16 ± 0.03	0.03 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.32 ± 0.01	0.06 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Liver	0.22 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Adrenals	0.32 ± 0.05	0.05 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Kidney	6.03 ± 1.08	0.84 ± 0.35	0.42 ± 0.06	0.56 ± 0.12	0.32 ± 0.02	0.30 ± 0.06
Lung	0.56 ± 0.16	0.07 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Heart	0.42 ± 0.05	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.20 ± 0.02	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Stomach	0.27 ± 0.05	0.11 ± 0.06	0.36 ± 0.45	0.15 ± 0.21	0.05 ± 0.03	0.02 ± 0.01
Intestine	0.23 ± 0.03	0.18 ± 0.20	0.36 ± 0.27	0.28 ± 0.27	0.01 ± 0.00	0.01 ± 0.00
Colon	0.13 ± 0.03	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.24 ± 0.08	0.11 ± 0.06
Testes	0.14 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skin	0.54 ± 0.03	0.09 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.21 ± 0.02	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.65 ± 0.07	0.08 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Brain	0.04 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fat	0.46 ± 0.10	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
Femur	0.25 ± 0.02	0.06 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Table 2. Radioactivity uptake in selected organs and tissues of rats after intravenous administration of 111In-DOTA-MG-45 and the effect of CCK-2 receptor blockade (animals were pretreated with the same peptide 0.1 mg/kg of body weight 15 min before the radiolabelled peptide dosing) 120 min after administration. The results are expressed as the percentage of injected dose per gram of the tissue (mean ± SD of four animals).

^{111}In-MG 45 %D/g	5 min	60 min	120 min	120 min	24 h	48 h
Blood	0.98 ± 0.12	0.09 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Plasma	1.67 ± 0.17	0.15 ± 0.02	0.02 ± 0.00	0.03 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.51 ± 0.07	0.05 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.010 ± 0.00
Liver	0.45 ± 0.07	0.29 ± 0.04	0.10 ± 0.01	0.03 ± 0.00	0.12 ± 0.02	0.33 ± 0.02
Adrenals	0.30 ± 0.03	0.06 ± 0.04	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.02
Kidney	7.76 ± 1.58	1.53 ± 1.53	0.58 ± 0.14	0.72 ± 0.24	0.34 ± 0.07	0.30 ± 0.08
Lung	0.83 ± 0.17	0.37 ± 0.04	0.31 ± 0.20	0.03 ± 0.00	0.06 ± 0.04	0.04 ± 0.03
Heart	0.43 ± 0.08	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.27 ± 0.05	0.09 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.08 ± 0.01	0.14 ± 0.03
Stomach	0.33 ± 0.10	0.08 ± 0.02	0.43 ± 0.57	0.02 ± 0.00	0.05 ± 0.01	0.03 ± 0.01
Intestine	0.26 ± 0.03	0.10 ± 0.03	0.69 ± 0.55	0.08 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Colon	0.12 ± 0.02	0.02 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.30 ± 0.15	0.12 ± 0.14
Testes	0.10 ± 0.01	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skin	0.48 ± 0.04	0.07 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.20 ± 0.03	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.64 ± 0.06	0.07 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.02
Brain	0.04 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fat	0.35 ± 0.09	0.04 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Femur	0.19 ± 0.03	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Table 3. Radioactivity uptake in selected organs and tissues of rats after intravenous administration of ^{111}In-DOTA-MG-47 and the effect of CCK-2 receptor blockade (animals were pretreated with the same peptide 0.1 mg/kg of body weight 15 min before the radiolabelled peptide dosing) 120 min after administration. The results are expressed as the percentage of injected dose per gram of the tissue (mean ± SD of four animals).

^{111}In-MG 47 %D/g	5 min	60 min	120 min	120 min premedication	24 h	48 h
Blood	1.45 ± 0.40	0.08 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Plasma	2.45 ± 0.67	0.13 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.57 ± 0.18	0.05 ± 0.02	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Liver	0.35 ± 0.09	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.02 ± 0.00
Adrenals	0.53 ± 0.16	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kidney	12.28 ± 7.43	1.84 ± 1.40	0.52 ± 0.07	0.49 ± 0.15	0.47 ± 0.14	0.44 ± 0.23
Lung	0.82 ± 0.21	0.06 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Heart	0.70 ± 0.24	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.31 ± 0.11	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Stomach	0.37 ± 0.07	0.12 ± 0.05	0.13 ± 0.06	0.04 ± 0.05	0.06 ± 0.01	0.04 ± 0.01
Intestine	0.38 ± 0.11	0.08 ± 0.03	0.15 ± 0.08	0.15 ± 0.13	0.013 ± 0.003	0.01 ± 0.00
Colon	0.20 ± 0.05	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.21 ± 0.04	0.23 ± 0.26
Testes	0.18 ± 0.04	0.04 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skin	0.77 ± 0.23	0.07 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Muscle	0.33 ± 0.09	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	1.04 ± 0.43	0.07 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01
Brain	0.06 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fat	0.67 ± 0.24	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Femur	0.34 ± 0.11	0.04 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00

Table 4. Radioactivity uptake in selected organs and tissues of rats after intravenous administration of ^{111}In-DOTA-MG-48 and the effect of CCK-2 receptor blockade (animals were pretreated with the same peptide 0.1 mg/kg of body weight 15 min before the radiolabelled peptide dosing) 120 min after administration. The results are expressed as the percentage of injected dose per gram of the tissue (mean ± SD of four animals).

^{111}In-MG 48 %D/g	5 min	60 min	120 min	120min premedication	24 h	48 h
Blood	0.64 ± 0.14	0.06 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Plasma	1.18 ± 0.26	0.10 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Pancreas	0.19 ± 0.04	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.17 ± 0.04	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Adrenals	0.20 ± 0.04	0.04 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Kidney	6.73 ± 2.51	2.14 ± 0.88	1.41 ± 0.36	1.62 ± 0.10	1.29 ± 0.23	1.32 ± 0.17
Lung	0.38 ± 0.08	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Heart	0.28 ± 0.06	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.17 ± 0.03	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Stomach	0.23 ± 0.08	0.37 ± 0.30	0.39 ± 0.34	1.13 ± 1.52	0.09 ± 0.03	0.07 ± 0.02
Intestine	0.18 ± 0.04	0.32 ± 0.32	0.66 ± 0.60	2.43 ± 3.25	0.01 ± 0.00	0.01 ± 0.01
Colon	0.08 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.23 ± 0.32	0.23 ± 0.07	0.14 ± 0.09
Testes	0.07 ± 0.02	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Skin	0.25 ± 0.03	0.05 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.11 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.49 ± 0.12	0.05 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Brain	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Fat	0.20 ± 0.07	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Femur	0.19 ± 0.05	0.14 ± 0.01	0.26 ± 0.38	0.06 ± 0.02	0.03 ± 0.01	0.03 ± 0.00

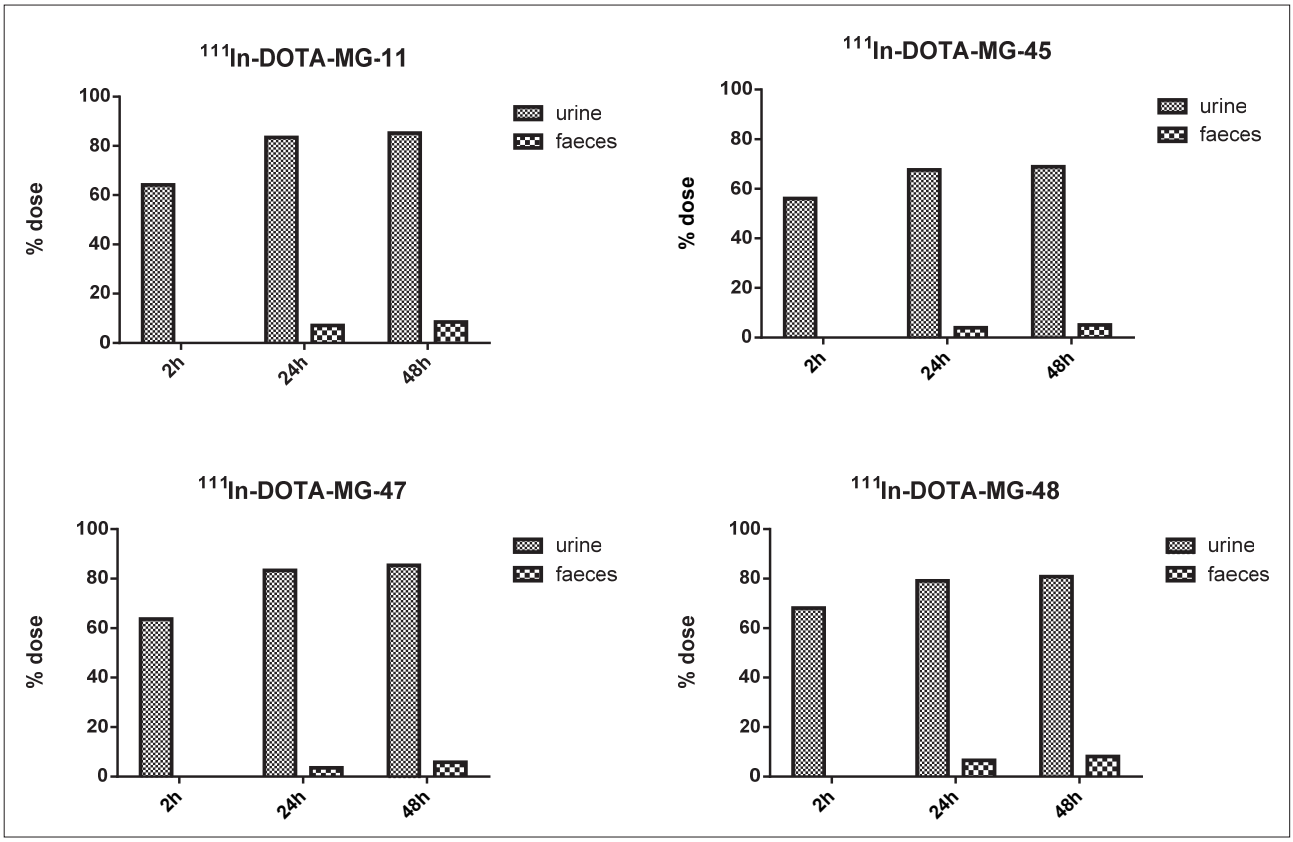

Fig. 4. Cumulative excretion of radioactivity in rats after intravenous administration of ^{111}In-DOTA minigastrins under study.

The internalization properties of the radiopeptides studied in CCK-2 receptor bearing AR42J cells suggest that all gastrin analogues under study had relatively rapid internalization in the early stages. At intervals longer than 60 min, the internalized fraction rise only slowly. Nevertheless, the results revealed some differences among the ^{111}In-DOTA minigastrins. The fastest and highest uptake into the cells was found for ^{111}In-DOTA-MG47. Simultaneously, there was a relatively small uptake of radioactivity in the kidneys. Very good results were also obtained for ^{111}In-DOTA-MG11. These two peptides thus seem to be the most promising agents of the tested compounds. The structural composition of the peptide, its lipophilicity, stability and also different affinity to CCK-2 receptors and the efficiency of receptor-mediated internalization of radiopeptide may be responsible for inter-drug differences in cellular internalization profile of radioactivity.

Biodistribution studies of ^{111}In-DOTA-minigastrins in normal rats revealed rapid blood clearance of radioactivity that became almost undetectable 2 hours postinjection and predominant excretion via the urinary tract. *In vivo* CCK-2 blockade with excess of non-radiolabelled DOTA-minigastrins resulted in a several fold reduction of radioactivity uptake in the stomach. These differences were not statistically significant however, due to large inter-individual variability most likely caused by the different number of CCK-2 receptors in this organ in individual animals.

High radioactivity uptake and retention by the kidneys is the main obstacle for clinical use of radiolabelled peptides, particularly for radionuclide therapy because of potential radionephrotoxicity[2,13]. The present results showed that without the penta-*D*Glu motif ^{111}In-DOTA-MG11, had relatively low radioactivity retention in the rat kidney. Renal radioactivity uptake after ^{111}In-DOTA-MG45 and ^{111}In-DOTA-MG47 were, however, only slightly higher than that of ^{111}In-DOTA-MG11. On the other hand, the kidney uptake of ^{111}In-DOTA-MG48 was high at all time points. Contrary to radiolabelled somatostatin analogues, taken up in the kidney via the megalin-cubilin system driven by positive charges, renal reabsorption of radiolabelled minigastrins is driven by negative charges[14]. This means that different mechanisms play a role in renal reabsorption of various radiolabelled peptides. Moreover, experiments in the guinea-pig kidney suggest the presence of CCK-2 receptors in the kidney, mostly over the cells of the distal collecting duct and to a much smaller degree over the glomeruli[15]. This means that at least some radioactivity in the kidney may be mediated by specific binding of the peptides to CCK-2 receptors.

The results of this preclinical study with ^{111}In-DOTA-chelated minigastrin analogues seemed encouraging; agents used in the present study showed a saturable affinity for CCK-2 receptors while internalization in a CCK-2 receptor-positive AR42J cell line was also demonstrated. In animal studies, rapid blood radioactivity wash-out, rapid renal clearance and saturable uptake of radioactivity in the CCK-2 receptor-positive stomach were observed. A relatively low radioactivity uptake was observed in the

kidney, with ^{111}In-DOTA-MG48 standing out as an exception to this.

ACKNOWLEDGEMENTS

The study was supported by the Grant Agency of the Czech Republic – grant No. P304/10/1738. The authors would like to thank Mrs. Jarmila Hoderova and Mrs. Eva Teichmanova for their excellent technical assistance.

Authorship contributions: All authors contributed equally to preparing the manuscript.

Conflict of interest statement: The authors stated that there are no conflicts of interest regarding the publication of this article.

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