Biodistribution and Elimination Characteristics of Two ^{111}In-labeled CCK-2/gastrin Receptor-specific Peptides in Rats

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Abstract. *Background: Due to their high CCK-2/gastrin receptor selectivity, high affinity, and rapid background clearance, radiolabeled minigastrins (MG) are emerging as promising new tools in the diagnosis and therapy of CCK-2/gastrin receptor-positive tumors. In this study, the pharmacokinetic profile, particularly the excretion mode, of two ^{111}In-labeled minigastrins was compared in rats. The first tracer, ^{111}In-MG-0 is based on (D)Glu^{1}-MG, while the second, ^{111}In-MG-11, is its des-(Glu)$_5$-derivative, expected to be less retained in renal tissue. Materials and Methods: The fate of ^{111}In-MG-0 and ^{111}In-MG-11 in the body of rats was investigated during biodistribution and bioelimination experiments, while the respective elimination parameters were determined in perfused rat liver and kidney models. Results: During biodistribution both compounds were rapidly cleared from the blood and most non-target organs whereas activity levels in the bowel and stomach declined slowly. The overall contribution of hepatobiliary excretion of ^{111}In-MG-0 and ^{111}In-MG-11 was relatively small. In the perfused rat liver their elimination into the bile was negligible. In contrast, renal excretion was the major excretion pathway for both analogs, mainly via glomerular filtration. However, kidney levels were substantially higher and retention was more prolonged in the case of ^{111}In-MG-0 as compared to ^{111}In-MG-11. Conclusion: The presence of the (Glu)$_5$-chain in ^{111}In-MG-0 appears to be implicated in the prolonged radioactivity retention in the kidney of rats.*

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The somatostatin analog ^{111}In-DTPA-octreotide (Octreoscan®) is used nowadays in the diagnosis of somatostatin receptor-positive neuroendocrine tumors (1-3). In addition to somatostatin receptors, other peptide receptors also seem to be clinically relevant targets in the diagnosis and therapy of malignant tumors. The cholecystokinin (CCK) receptor is recently regarded as another potentially useful target for nuclear oncology applications (2, 4). Cholecystokinin receptors comprise two distinct subtypes: the CCK-1 and the CCK-2/gastrin receptor subtype (4, 5), characterized by their different binding affinity toward known agonists and antagonists (6, 7). The CCK-1 receptors have been identified in normal tissues, such as in the smooth muscle of the gallbladder and of the sphingter of Oddi, the pancreatic acini and the brain (4, 8, 9). With some exceptions, CCK-1 receptors are rarely expressed in human tumors (10). CCK-2/gastrin receptors have been found on gastric parietal cells, gastrointestinal smooth muscle cells, in the brain, and in selected areas of the kidney (4, 6, 7). CCK-2/gastrin receptors have also been shown to be expressed in medullary thyroid cancers (MTC), small cell lung cancers (SCLC), stromal ovarian cancers, astrocytomas (11-13) and in several other types of human tumors (14, 15).

Radiopharmaceuticals developed for the targeting of CCK-2/gastrin receptor-positive tumors with ^{111}In and therapeutic radionuclides, such as ^{90}Y, are either minigastrin (16, 17), or non-sulfated CCK octapeptide (CCK-8) derivatives linked to diethylenetriaminepentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecane-N,N',N'', N'''-tetraacetic acid (DOTA) (18). ^{99m}Tc-labeled [(D) Glu^{1}]MG analogs modified with acyclic tetraamines were proposed for the CCK-2/gastrin receptor-targeted diagnosis of human MTC (19). Pilot clinical studies demonstrated that the majority of primary and metastatic MTC lesions can be visualized by CCK-2/gastrin receptor scintigraphy (16, 17, 19-21). In addition, CCK-2/gastrin receptor-targeted

radiotherapy of MTC using radiolabeled minigastrin was reasonably successful in a small number of patients (16, 17, 20). However, the high radiation dose to the kidney due to the prolonged renal retention remains a major concern and minigastrin analogs with reduced renal retention need to become available. Although premedication with polyglutamate chains of at least 5 glutamic acid residues was showed to effectively reduce the undesirable renal accumulation of radiolabeled minigastrins in mice (22), the effectiveness of this strategy in human has not yet been proven. In a study on the effects of charge and (Glu) x chain length in a library of ^{111}In-DTPA-(D)Glu^1-minigastrins on renal retention, biokinetics and CCK-2/gastrin receptor-targeting capacity, ^{111}In-MG-11, altogether lacking the $(Glu)_5$-chain of minigastrin, emerged as the agent of choice for further clinical validation (23).

In the present work, the pharmacokinetic profile of ^{111}In-DTPA-(D)Glu^1-minigastrin (^{111}In-MG-0) was compared with that of the newly proposed truncated analog ^{111}In-MG-11 in healthy rats. Special attention has been paid to the renal handling of these two analogs, which are predominantly excreted *via* the kidneys. The study includes biodistribution and bioelimination experiments in rats as well as experiments employing perfused rat liver and kidney models.

Materials and Methods

Radiolabeling and radiochemical analysis. Minigastrin 0 (MG-0, DTPA-*D*-Glu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2) and minigastrin 11 (MG-11, DTPA-*D*-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2) were prepared as reported elsewhere (23). Polyfructosan (INUTEST) (Laevosan, Linz, Austria) was used as a marker of glomerular filtration rate and the sodium salt of bromosulfophthalein as a marker of the liver function. All other chemicals were of analytical grade and were purchased from Sigma-Aldrich Co. (Steinheim, Germany).

To 10 μl of peptide solution (1 μg/μl in 0.5M acetate buffer pH 5.4), 20 μl of 0.5 M acetate buffer (pH 5) was added together with the same volume of ~40 MBq $^{111}InCl_3$ in 0.04 M HCl (Amersham, UK). After 30 min incubation at room temperature, HPLC analysis on a Pharmacia LKB system efficient with a LichChroCart RP C18, 125 mm x 4 mm (5 μm) column (Merck, Darmstadt, Germany) was carried out using the following mobile phases: 0.01 phosphate buffer pH 6.5 (phase A) and acetonitrile (phase B). Elution gradient during analysis was as follows: 0-5 min 0% B; 5-35 min 0-30% B; 35-55min 30% B at a flow rate of 1 ml/min. Radiochemical purity of the peptides was always higher than 98%.

Distribution and elimination studies in vivo. Male Wistar rats weighing 200-220 g were used for the experiments. The tracers were administered into the tail vein in a volume of 0.2 ml (approx. 1 μg/kg of body weight; activity 0.5-1 MBq/kg). At the selected time interval (5 min, 1 h, 2 h, 24 h or 48 h) post injection (pi), a blood sample was collected in a glass tube containing dry heparin. After exsanguination, selected organs were removed to determine the distribution of ^{111}In activity with time. For bioelimination studies, radiopharmaceuticals were administered as described above and each animal was placed in a separate glass metabolic cage. Urine and faeces were collected at 2 h (urine only), 24 h and 48 h pi. Rats were compelled to empty their urinary bladders by handling (immobilization).

The Ethical Committee of the Faculty of Pharmacy of Charles University, approved the protocols of animal experiments. These protocols were pursued in accordance with the directive of the Ministry of Education of the Czech Republic.

Rat kidney perfusion. Male Wistar rats weighing 280-320 g were used for perfusion experiments, as described elsewhere (24). In brief, the kidney was perfused with 100 ml Krebs-Henseleit solution containing 6% bovine serum albumin, 5-6% washed rat erythrocytes and a mixture of amino acids and metabolic substrates. The perfusion was performed under a recirculation regimen at a constant arterial pressure. The agents under study were added into the perfusion system as a bolus (0.2 μg). Polyfructosan was used to determine the glomerular filtration rate. Concentrations of polyfructosan in the perfusate and the urine were measured colorimetrically (25). The elimination profiles in the perfused rat kidney were characterized on the basis of total renal clearance (urinary activity x volume of urine/activity in perfusate) and filtration clearance (= elimination rate due to glomerular filtration) calculated as follows: glomerular filtration rate x free fraction of the agent in the perfusate. Radioactivity in the kidney and ratio of kidney to perfusate radioactivity at the end of the perfusion were also calculated

Rat liver perfusion. The recirculation perfusion study was performed as described elsewhwre (26). Under pentobarbital anesthesia, the bile duct and the portal vein of the rat were cannulated. The blood was washed out from the liver with Krebs-Henseleit buffer (pH 7.4 with glucose) *via* the inferior vena cava and the perfusion medium (oxygenated Krebs-Henseleit buffer, pH 7.4, supplemented with 10 mM glucose, 4% BSA and 10% washed bovine erythrocytes) was infused into the portal vein. The flow of the perfusate medium was kept at 25 ml/min and the radiotracer was added to a reservoir. The experiment was performed for 90 min after drug administration and samples from the inflow and outflow perfusate and bile were collected throughout the experiment. Hepatobiliary clearance was calculated as the activity of the agent eliminated into the bile per min/the activity in 1 ml perfusate. In all experiments, ^{111}In activity in collected samples was measured in a 1480 Wizard 3 gamma-spectrometer (Wallac, Finland) and related to appropriate standards.

Results

Distribution and elimination studies in rats. Cumulative data of ^{111}In-MG-0 and ^{111}In-MG-11 biodistribution in the rat are summarized in Table I and II, respectively, and are expressed as % injected dose/organ (% ID/organ) and as % injected dose/g (% ID/g) for the 5 min, 1, 2, 24 and 48 h pi time intervals. Both tracers were rapidly cleared from blood *via* the kidneys and the urinary system. However, ^{111}In-MG-11, lacking the Glu_5-sequence, was more rapidly excreted into the urine with only a small part of the radioactivity retained in renal tissue (<2.0% at 1 h pi). In contrast, non-

Table I. *Distribution of ^{111}In-MG-0 in rats (mean±SD, n=4).*

	5 min	1 h	2 h	24 h	48 h
% injected dose in whole organ (% ID/organ)					
Liver	1.845±0.882	0.287±0.171	0.105±0.021	0.074±0.004	0.070±0.008
Stomach	1.310±0.575	0.964±0.329	0.606±0.215	0.380±0.034	0.209±0.024
Intestine	2.191±0.884	4.080±6.884	1.604±1.702	0.412±0.272	0.085±0.029
Colon	1.102±0.462	0.207±0.096	0.051±0.017	3.513±1.229	0.762±0.292
Kidney	32.414±11.105	47.039±26.550	31.453±3.418	33.542±2.678	29.581±5.048
Lung	0.818±0.341	0.106±0.071	0.016±0.008	0.006±0.003	0.005±0.001
Heart	0.363±0.172	0.031±0.013	0.005±0.002	0.002±0.000	0.002±0.001
% injected dose per g (% ID/g)					
Blood	1.341±0.638	0.114±0.064	0.010±0.004	0.001±0.000	0.001±0.000
Stomach	0.705±0.312	0.468±0.068	0.300±0.121	0.219±0.033	0.126±0.021
Liver	0.284±0.136	0.045±0.024	0.018±0.004	0.012±0.002	0.011±0.001
Pancreas	0.415±0.185	0.058±0.027	0.012±0.003	0.009±0.002	0.006±0.003
Adrenals	0.451±0.183	0.242±0.242	0.016±0.004	0.010±0.009	0.007±0.007
Kidney	19.224±7.681	26.428±14.632	18.507±2.316	20.358±1.040	17.336±2.086
Thyroid	0.873±0.403	0.080±0.032	0.009±0.008	0.003±0.004	0.003±0.005
Muscle	0.264±0.130	0.028±0.018	0.004±0.001	0.002±0.001	0.001±0.000

Table II. *Distribution of ^{111}In-MG-11 in rats (mean±SD, n=4).*

	5 min	1 h	2 h	24 h	48 h
% injected dose in whole organ (% ID/organ)					
Liver	1.447±0.142	0.186±0.032	0.101±0.016	0.069±0.008	0.057±0.007
Stomach	1.519±1.569	0.261±0.069	0.196±0.056	0.116±0.018	0.291±0.461
Intestine	2.777±1.969	0.487±0.092	0.569±0.172	0.150±0.054	0.040±0.023
Colon	0.967±0.137	0.119±0.003	0.049±0.019	3.189±2.406	0.678±0.497
Kidney	12.281±8.745	1.932±0.254	1.660±0.110	1.472±0.087	1.386±0.097
Lung	0.698±0.055	0.085±0.039	0.024±0.014	0.005±0.001	0.004±0.001
Heart	0.245±0.142	0.028±0.008	0.005±0.001	0.002±0.000	0.001±0.000
% injected dose per g (% ID/g)					
Blood	0.993±0.043	0.076±0.015	0.010±0.003	0.001±0.000	0.000±0.000
Stomach	0.575±0.446	0.124±0.032	0.083±0.027	0.065±0.014	0.125±0.174
Liver	0.218±0.011	0.028±0.003	0.015±0.002	0.010±0.002	0.009±0.001
Pancreas	0.323±0.021	0.038±0.005	0.011±0.001	0.007±0.001	0.006±0.000
Adrenals	0.307±0.034	0.037±0.006	0.013±0.003	0.003±0.003	0.005±0.004
Kidney	7.043±4.910	1.105±0.187	0.893±0.106	0.838±0.089	0.823±0.082
Thyroid	0.707±0.015	0.050±0.029	0.012±0.004	0.007±0.002	0.003±0.002
Muscle	0.192±0.015	0.018±0.006	0.003±0.001	0.002±0.001	0.002±0.000

truncated ^{111}In-MG-0 showed high uptake and prolonged retention in the rat kidney with ~30% ID still detected in renal tissue at 48 h pi.

Fast uptake of both minigastrins in the stomach (known to naturally express CCK-2/gastrin receptors) was evident as soon as 5 min pi. However, ^{111}In-MG-11 was quickly lost from the stomach while the decrease of stomach radioactivity was slower in the case of ^{111}In-MG-0. In CCK-1 receptor-positive organs (*e.g.* pancreas) and in CCK-2/gastrin receptor-negative organs (*e.g.* adrenals, heart) radioactivity values paralleled blood clearance. The radioactivity detected in the small and large intestine after injection of both analogs was attributed to minor excretion *via* the gastrointestinal tract. The uptake in all other organs and tissues studied (spleen, testes, brain, femur, skin, abdominal fat) remained below 0.5% ID at all time intervals. From the radioactivity concentration values (% ID/g values in Table I and II) high and prolonged uptake was evident in the kidneys and stomach for both compounds. Thyroid uptake, an important factor of the

Table III. *Cumulative excretion (% injected dose) of ^{111}In-MG-0 and ^{111}In-MG-11 after i.v. administration in rats (mean±SD, n=4).*

Compound	2 h		24 h		48 h	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
^{111}In-MG-0	53.88±4.81	–	57.09±5.52	3.42±1.69	59.05±4.68	4.76±1.02
^{111}In-MG-11	78.22±7.99	–	80.54±6.96	4.86±3.09	80.96±6.81	6.48±3.72

Table IV. *Excretion and retention data of ^{111}In-MG-0 and ^{111}In-MG-11 in perfused rat kidney (values are means±SD, n=4).*

Compound	Renal clearance (ml/min/g)	Free fraction in perfusate	Filtration clearance (ml/min/g)	Radioactivity of the kidney at the end of the perfusion (% dose)	Kidney-to-perfusate ratio at the end of the perfusion
^{111}In-MG-0	0.418±0.034	0.523±0.022	0.358±0.049	13.4±0.9	20.6±2.0
^{111}In-MG-11	0.599±0.033	0.829±0.089	0.612±0.082	2.0±0.4	3.2±0.4

feasibility to image primary MTC in the thyroid, although initially substantial, declined very rapidly thereafter. Cumulative excretion data in rat urine and faeces during the 48 h span is presented in Table III. Urine excretion was the main elimination pathway for both ^{111}In-labeled minigastrins and most of the radioactivity was eliminated during the first 2 h. It is worth noticing that the percentage of radioactivity found in the urine was markedly higher for ^{111}In-MG-11 as compared with ^{111}In-MG-0.

Rat kidney perfusion. The renal clearance parameters and the accumulation characteristics of ^{111}In-MG-0 and ^{111}In-MG-11 in the perfused rat kidney along with their free fractions in the perfusate are presented in Table IV. No significant differences in the renal clearance values were found during 60 min of perfusion. From the corresponding renal elimination characteristics, it is apparent that ^{111}In-MG-0 was more slowly filtered in the glomeruli than ^{111}In-MG-11, despite its significantly higher accumulation in renal tissue.

Rat liver perfusion. Rat liver perfusion experiments revealed that the excretion rate by the liver into the bile was very low for ^{111}In-MG-11 and negligible for ^{111}In-MG-0 (Table V). Liver-to-perfusate ratios at the end of perfusion experiments were relatively low for both agents and indicated that they were little accumulated in liver cells.

Table V. *Excretion parameters of ^{111}In-MG-0 and ^{111}In-MG-11 in perfused rat liver (values are means±SD, n=4).*

Compound	Bile clearance of the peptide (ml/min)	Liver-to-perfusate ratio at the end of the perfusion
^{111}In-MG-0	0.0002±0.0001	0.253±0.109
^{111}In-MG-11	0.0034±0.0008	0.200±0.016

Discussion

Radiolabeled CCK- or gastrin-peptide analogs have been proposed as potential diagnostic or therapeutic drugs against CCK-2/gastrin receptor-expressing tumors, such as MTC or SCLC (16-23). Previous studies have revealed the impact of a $(Glu)_5$-sequence in radiolabeled minigastrins on the prolonged radioactivity retention in the kidneys of experimental animals and patients (22, 23). As a result, the anionic pentaglutamate sequence of minigastrins leads to unfavourably high radiation doses to the kidney. This disadvantage seems to be compensated for, by the higher CCK-2/gastrin receptor targeting capacity of minigastrins both *in vitro* and *in vivo versus* the respective CCK analogs. In the present study, we compared the distribution and elimination profile in rats of two such ^{111}In-labeled peptide analogs derived either from minigastrin, ^{111}In-MG-0, or from *des*-$(Glu)_5$-minigastrin, ^{111}In-MG-11. The latter was recently selected from an ^{111}In-labeled DTPA-(D)Glu^1-MG library as the agent of choice for CCK-2/gastrin receptor-targeted tumor scintigraphy because it combines high affinity binding to the receptor with low uptake and retention in the kidneys of experimental animals (23).

Our results from the distribution and elimination studies in rats conducted in the present work confirmed the findings from previous reports. Thus, we were able to observe higher uptake and longer retention of ^{111}In-MG-0 in the CCK-2/gastrin receptor-rich stomach in comparison with ^{111}In-

MG-11. Although both tracers were excreted from blood and non-target tissues *via* the kidneys and the urinary system, they differed significantly in the amount of radioactivity accumulated in the kidney. Renal radioactivity levels of <2% ID at 1 h pi declined to 1.4% ID at 48 h pi for the *des*-$(Glu)_5$ analog, ^{111}In-MG-11. Conversely, in the case of ^{111}In-MG-0, nearly 30% ID levels were still detected in renal tissue at 48 h pi. These findings were corroborated by results from experiments in perfused rat kidney. Thus, renal retention of ^{111}In-MG-0 at 60 min perfusion was several times higher in comparison with ^{111}In-MG-11.

It is generally accepted that tubular reabsorption is responsible for the renal uptake of peptides (27-28). The mechanism of renal accumulation is usually explained as a process including several transport and metabolic steps. The accumulated radiolabeled compounds are primarily filtered in the glomeruli and subsequently partly reabsorbed into the cells of proximal tubules. Thereafter, the agents are transferred into lysosomes by means of pinocytosis wherein they are degraded by proteolytic enzymes. Most often, breakdown products, namely radiolabeled chelate-conjugated amino acids, cannot leave the lysosomes and remain trapped in the proximal tubular cells.

In the rat kidney perfusion model, we found differences in the total renal clearance rates as well as in the glomerular filtration clearance rates between non-truncated and truncated minigastrins. This difference was attributed to the different degree of radiotracer binding to plasma proteins found in the applied perfusate. The filtration clearance of both tracers was not significantly different from their total renal clearance. This means that the radiotracer amount filtered in the glomeruli is practically the same as the amount excreted. The decisive renal excretion process for both peptides seems to be glomerular filtration. It should be noted that the filtration clearance of ^{111}In-MG-0 was lower than the total renal clearance, consistent with a partial secretion of this agent in the renal tubular system. However, the difference between these clearance values was not significant. Moreover, tubular secretion has so far been shown only for smaller peptides, such as ^{99m}Tc-labeled tetrapeptides (24).

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