ORIGINAL ARTICLE

Albumin-derived peptides efficiently reduce renal uptake of radiolabelled peptides

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Abstract

Purpose In peptide-receptor radionuclide therapy (PRRT), the maximum activity dose that can safely be administered is limited by high renal uptake and retention of radio-labelled peptides. The kidney radiation dose can be reduced by coinfusion of agents that competitively inhibit the reabsorption of radiolabelled peptides, such as positively charged amino acids, Gelofusine, or trypsinised albumin. The aim of this study was to identify more specific and potent inhibitors of the kidney reabsorption of radiolabelled peptides, based on albumin.

Methods Albumin was fragmented using cyanogen bromide and six albumin-derived peptides with different numbers of electric charges were selected and synthesised. The effect of albumin fragments (FRALB-C) and selected albumin-derived peptides on the internalisation of ^{111}In-albumin, ^{111}In-minigastrin, ^{111}In-exendin and ^{111}In-octreotide by megalin-expressing cells was assessed. In rats, the effect of Gelofusine and albumin-derived peptides on the renal uptake and biodistribution of ^{111}In-minigastrin, ^{111}In-exendin and ^{111}In-octreotide was determined.

Results FRALB-C significantly reduced the uptake of all radiolabelled peptides in vitro. The albumin-derived peptides showed different potencies in reducing the uptake of ^{111}In-albumin, ^{111}In-exendin and ^{111}In-minigastrin in vitro.

The most efficient albumin-derived peptide (peptide #6), was selected for in vivo testing. In rats, 5 mg of peptide #6 very efficiently inhibited the renal uptake of ^{111}In-mini-gastrin, by 88%. Uptake of ^{111}In-exendin and ^{111}In-octreotide was reduced by 26 and 33%, respectively.

Conclusions The albumin-derived peptide #6 efficiently inhibited the renal reabsorption of ^{111}In-minigastrin, ^{111}In-exendin and ^{111}In-octreotide and is a promising candidate for kidney protection in PRRT.

Introduction

Excretion of radiolabelled hydrophilic peptides and small proteins occurs largely via the kidneys. In peptide-receptor radionuclide therapy (PRRT), rapid clearance of the radio-labelled peptides from the blood and low retention in the kidneys are important to minimise the radiation dose to normal tissues. However, as outlined in more detail previously [1], a part of the filtered load of these small proteins and peptides is reabsorbed from the ultrafiltrate in the proximal tubules and retained there, exposing the kidneys to a relatively high radiation dose. Indeed, acute and chronic kidney damage have been described in patients receiving PRRT and in animal models [2–5] and kidney toxicity is currently dose-limiting in PRRT [3, 4, 6]. Reduction of the renal radiation dose by reducing the renal retention of radiolabelled peptides facilitates the adminis-tration of higher activity doses in PRRT, which is expected to increase its therapeutic effect.

A possible approach to reducing the renal reabsorption of radiolabelled peptides is to interfere with endocytic

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receptors on the proximal tubular cells. The receptors involved in the tubular reabsorption of peptides have not yet been characterised completely, but for various peptides, involvement of megalin and cubilin has been shown [7–10]. Cubilin is a large extracellular receptor that depends on other receptors such as megalin for its internalisation. Megalin is a multiligand receptor belonging to the LDL receptor family. The receptor contains four large cysteine-rich ligand binding domains and is a high-capacity transport system with varying affinity for the reabsorption of a large series of structurally non-related compounds, such as albumin, vitamin D binding protein, β_2-microglobulin, aprotinin, Ca^{2+}, etc. [7, 11, 12]. Charges may play an important role in the binding of ligands to megalin [12, 13] or to other renal receptors. Gotthardt et al. found a positive correlation between the renal retention and the total number of charged amino acid residues of radiolabelled peptides, and suggested a causal relationship [14]. It has been shown that some ligands bind to megalin via their cationic sites and that co-administration of cationic compounds can inhibit the renal reabsorption of these ligands [8, 15]. Indeed, in patients undergoing PRRT with radiolabelled somatostatin analogues as well as in various animal models, the renal uptake of different radiolabelled peptides and antibody fragments can effectively be reduced by co-administration of basic amino acids [16–19]. However, these compounds can cause side-effects at high doses, such as nausea, hyperkalemia, arrhythmias and even nephrotoxicity [15, 18, 20]. Succinylated gelatin (Gelofusine, Gelo), a plasma expander that consists of a mixture of collagen-derived peptides, has also been shown to efficiently reduce the renal reabsorption of various radiolabelled peptides in animals, and of ^{111}In-octreotide in humans [1, 21–23]. Nevertheless, more efficient inhibitors are needed since none of these substances completely inhibits the reabsorption of radiolabelled peptides.

Albumin, the most abundant plasma protein, is a natural ligand of megalin and cubilin. Due to albumin's size and charge (67 kDa, predominantly anionic at physiological pH), only a small fraction of circulating albumin is filtered in the glomeruli [13, 24, 25]. This small fraction is subsequently reabsorbed by megalin/cubilin-mediated endocytosis. Previously, we showed that peptide fragments obtained by trypsinisation of bovine serum albumin (BSA) reach higher concentrations in the proximal tubules than intact albumin and are efficient inhibitors of the renal reabsorption of ^{111}In-octreotide, ^{111}In-exendin and ^{111}In-minigastrin in rats [1]. However, trypsin cleaves peptides at the carboxyl terminus of arginine and lysine residues [26]. Since BSA contains 78 arginine and lysine residues, its trypsinisation yields a complex mixture of many different albumin fragments. We hypothesised that some of these may not actually contribute to the blocking of the renal

reuptake of radiolabelled peptides, but might cause side effects. Better defined albumin fragments may be obtained by digestion of albumin by cyanogen bromide (CNBr) in formic acid. CNBr cleaves peptides at the carboxyl terminus of methionine residues and formic acid cleaves peptides at aspartic acid–proline bonds [27]. BSA has four methionine residues and two aspartic acid–proline bonds. Thus, digestion of BSA by CNBr in formic acid yields a maximum of 28 possible fragments.

Since megalin binds various structurally different peptides and proteins, we hypothesised that different fragments of albumin may bind to megalin and be potential candidates for inhibiting the renal reuptake of radiolabelled peptides. In addition to the mixture of CNBr-digested albumin fragments (FRALB-C), a set of albumin-derived peptides was selected. The selection of these peptides was based on the distribution of charged amino acid residues in BSA. We aimed to identify albumin fragments that potently inhibit the renal reabsorption of radiolabelled peptides, in order to maximise the inhibitory effect and to minimise potential side effects.

In this study, the effect of FRALB-C and a series of synthesised albumin-derived peptides on the renal uptake of [^{111}In-DTPA-d-Phe^1]-octreotide (^{111}In-octreotide), [Lys^{40}(a-minohexanoic acid-DTPA-^{111}In)NH$_2$]-exendin-3 (^{111}In-exendin) and [^{111}In-DOTA-Glu^1]-minigastrin (^{111}In-minigastrin) was analyzed, both in vitro and in rats.

Materials and methods

Fragmentation of albumin

Intact BSA (2.26 µmol (150 mg), Sigma) was added to 4.5 ml of 70% v/v formic acid with dithiothreitol (6.7 mM) and heated for 2 min at 100°C. Subsequently, 1.13 mmol of CNBr (Sigma) was added and the mixture was incubated for 24 h at room temperature in the dark, followed by 5 min of incubation at 50°C. The acid was neutralised by adding 9 ml of 4-ethylmorpholine (Sigma), after which the mixture was dialyzed against phosphate buffered saline (PBS). The fragmented albumin was analyzed by fast protein liquid chromatography (FPLC) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein concentration in the FRALB-C mixture was determined with the Bio-Rad Protein Assay using intact BSA as a reference.

Selection and synthesis of albumin fragments

Six parts of the BSA amino acid sequence were selected for peptide synthesis. The selection was based on the distribution of charged amino acid residues in these sequences and their net charge at physiological pH. Five peptides contain-

ing different combinations of positive and negative charges were selected and synthesised, each composed of 16 amino acid residues. An uncharged peptide was synthesised as well, but this was not studied since it could not be dissolved in water. A sixth peptide was synthesised, which was the smallest possible fragment formed after CNBr digestion of BSA, consisting of the 36 C-terminal amino acid residues. The characteristics of the synthesised peptides are summarised in Table 1. Peptides were synthesised by standard solid-phase F-moc chemistry and purified by high-performance liquid chromatography (HPLC) at Peptide Specialty Laboratories GmbH (Heidelberg, Germany) and at Peptide 2.0 (Chantilly, VA, USA).

Other compounds used to reduce renal reabsorption

Gelofusine (Gelo, Braun) is a solution of succinylated gelatin fragments (40 g/l). The molecular mass of these fragments in Gelo averages 30 kDa. Intact BSA was dissolved in PBS at a concentration of 100 g/l. Lysine solution was prepared as a 160 g/l solution in PBS.

Radiolabeling

[DTPA-d-Phe[1]]-octreotide (Octreoscan, Covidien) was labelled with ^{111}In by adding 37 MBq of ^{111}InCl$_3$ (Covidien) to 1 µg of Octreoscan and incubating for 30 min at room temperature. [Lys^{40}(Ahx-DTPA)NH$_2$]-exendin-3 (exendin, Peptide Specialty Laboratories GmbH, Heidelberg, Germany) was synthesised using standard F-moc chemistry. The peptide was labelled with ^{111}InCl$_3$ in a 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer, pH 5.5. A mixture of 19 MBq of ^{111}In and 1 µg of exendin was incubated for 30 min at room temperature. [DOTA-Glu^1]-minigastrin (minigastrin, Peptide Specialty Laboratories) was synthesised using standard F-moc chemistry. Minigastrin was labelled with ^{111}InCl$_3$ in a 0.25 M ammonium acetate buffer,

pH 5.5. ^{111}InCl$_3$ (22.2 MBq) was added to 1 µg of minigastrin and the mixture was incubated at 95°C for 25 min.

BSA was conjugated with DTPA as described by Hnatowich et al. [28] and labelled with ^{111}In by adding 11 MBq of ^{111}InCl$_3$ to 1 µg of DTPA-BSA in a 0.25 M ammonium acetate buffer, pH 5.5 (100 µl) and incubating for 20 min at room temperature. Radiochemical purity and labeling efficiency of the labelled peptides and proteins were determined by silica gel instant thin layer chromatography and reverse phase HPLC.

In vitro studies

Rat yolk sac epithelial (BN16) cells, expressing megalin and cubilin [29, 30] were kindly provided by Prof. P.J. Verroust. Cells were cultured at 37°C in BN16 medium, consisting of Dulbecco's modified Eagle's medium plus 4.5 g/l glucose (Invitrogen) with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen).

The potency of FRALB-C to inhibit the interaction of the radiolabelled peptides with BN16 cells was determined in vitro in triplicate. The cells were seeded into 6-well plates at 3×10^5 cells/well and cultured until confluent. The cells were washed with PBS and incubated for 1 h with serum-free BN16 medium. The cells were washed again with PBS, and 2 ml of Ringer's solution (Braun) with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, pH 7.4 was added. Subsequently, 100 µl of the inhibitor was added: either PBS, Gelo or 100 µg of FRALB-C. Directly after addition of the inhibitor, 80 kBq (50 µl) of ^{111}In-albumin, ^{111}In-octreotide, ^{111}In-exendin or ^{111}In-minigastrin was added. After 1 h of incubation at 37°C, cells were rinsed with Ringer's solution and harvested using cotton swabs. Radioactivity was measured in a shielded well-type gamma counter (Wizard, Wallac). To study the inhibitory potency of the albumin-derived peptides, the same procedure was used. Increasing doses of the six peptides (10, 30 and

Table 1 Characterisation of albumin-derived peptides*

#	Amino acid sequence	Number of charged AA residues at pH 7			Net charge at pH 7	Iso-electric point	Molecular mass (Da)	Location in BSA (AA numbers)
		+	-	total				
1	DEKKFWGKYLYEIARR	5	3	8	+2	10.4	2103	129-144
2	SSARQRLRCASIQKFG	4	0	4	+4	12.3	1809	191-206
3	DKPLLEKSHCIAEVEK	4	4	8	0	4.7	1840	279-294
4	DAIPENLPPLTADFAE	0	4	4	-4	2.7	1714	295-310
5	ALTPDETYVPKAFDEK	2	4	6	-2	4.2	1825	489-504
6	ENFVAFVDKCCAADDKEA CFAVEGPKLVVSTQTALA	3	6	9	-3	4.5	3792	548-583

*Characteristics of the albumin-derived peptides that were synthesised and studied in our in vitro assay. AA = amino acid. Positively and negatively charged amino acid residues are printed in red and blue, respectively.

100 μg per incubation) were compared with the potency of intact albumin (10 mg) and Gelo (5 mg) to reduce the binding of ^{111}In-albumin, ^{111}In-exendin and ^{111}In-minigastrin. Internalisation of these radiolabelled compounds by BN16 cells was studied using essentially the same assay. Cells were incubated with ^{111}In-albumin, ^{111}In-exendin or ^{111}In-minigastrin for 1 h at 37°C or 0°C. Internalised activity and cell-surface bound activity was determined by incubating the cells for 10 min in 1 ml 0.1 M acetic acid, 154 mM NaCl, pH 2.8 at 0°C. The activity in the supernatant (cell-surface bound) and the activity in the cells (internalised) was determined.

Animal studies

Groups of five male Wistar rats (200–220 g) were used in all experiments. The potency of FRALB-C to inhibit the renal reabsorption of ^{111}In-octreotide was compared with the reference agents PBS, lysine and Gelo. The animals were injected i.v. with 0.5 ml of the inhibitor (either PBS, lysine (80 mg), Gelo (20 mg) or FRALB-C (1 mg, 5 mg or 20 mg)) via the tail vein. One minute later, ^{111}In-octreotide was injected i.v. (2 MBq/rat).

The animals were killed 20 h after injection and a blood sample was drawn and organs were dissected. The kidneys, adrenals, spleen and stomach, as well as samples of muscle, lung, pancreas, liver and duodenum were harvested. The tissues were weighed and counted in a shielded well-type gamma counter together with the injection standards. Radioactivity concentration was expressed as percentage of the injected dose per gram tissue (% ID/g).

The albumin-derived peptide which most potently reduced the binding of ^{111}In-minigastrin and ^{111}In-exendin in the cell binding assay, peptide #6, was studied in rats according to the same procedure. The effect of peptide #6 (0.2 mg, 1 mg or 5 mg per rat) on the uptake of ^{111}In-minigastrin, ^{111}In-exendin and ^{111}In-octreotide was studied and compared to PBS and Gelo (20 mg per rat) as negative and positive control, respectively.

Statistical analysis

Data are presented as mean % reduction ± standard deviation. The analysis of differences in cell binding and kidney uptake was performed using Student's t-test. Biodistribution data in other organs were analyzed by one-way ANOVA. The significance level was set at 0.05.

Results

Radiolabelling

Radiochemical purity and labelling efficiency of all compounds were higher than 95%.

In vitro studies

The internalisation assay showed that at 37°C, >90% of cell-associated ^{111}In-albumin and ^{111}In-minigastrin and >75% of ^{111}In-exendin was internalised (data not shown). This indicates that these radiolabelled compounds were rapidly internalised by the BN16 cells.

As summarised in Fig. 1, FRALB-C significantly reduced the uptake of all radiolabelled peptides in BN16 cells. A dose of 100 μg of FRALB-C per incubation reduced the uptake of ^{111}In-albumin by 75%±5.7% ($p<0.001$, Fig. 1a). The uptake of ^{111}In-minigastrin, -exendin and -octreotide was reduced by 95%±6.3% ($p<0.001$), 67%±6.9% ($p<0.001$) and 39%±7.4% ($p<0.01$), respectively (Fig. 1b–d). The lower doses of 30 μg and 10 μg FRALB-C were less effective (data not shown). Gelofusine (4 mg) significantly reduced the uptake of all radiolabelled peptides as well.

The effects of 100 μg of the six albumin-derived peptides on the uptake of ^{111}In-albumin, -minigastrin and -exendin in BN16 cells are summarised in Fig. 2. The uptake of ^{111}In-albumin (Fig. 2a) was inhibited significantly (all $p<$
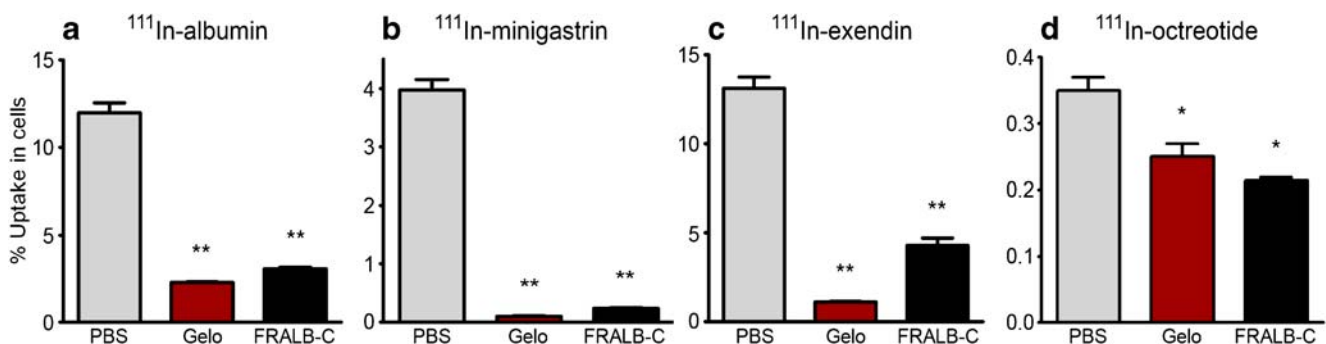

Fig. 1 Inhibition of the uptake of ^{111}In-albumin (**a**), ^{111}In-minigastrin (**b**), ^{111}In-exendin (**c**) and ^{111}In-octreotide (**d**) in BN16 cells by Gelo (4 mg) or FRALB-C (100 μg). Results are presented as mean % bound activity; *error bars* indicate standard error of the mean (SEM). *$P<0.05$; **$P<0.005$

0.003) by peptide #1 (54%±9.3%), peptide #3 (50%± 9.1%), peptide #5 (37%±11%) and most potently by peptide #6 (75%±9.1%), as well as by intact albumin (91%±9.1%) and Gelo (65%±9.0%).

As depicted in Fig. 2b, the uptake of ^{111}In-minigastrin was reduced significantly by peptide #3 (60%±6.0%, $p=$ 0.002), peptide #4 (76%±4.5%, $p=0.0002$), peptide #5 (37%±5.7%, $p=0.005$) and peptide #6 (96%±4.3%, $p<$ 0.0001). Albumin and Gelo also significantly reduced the uptake, by 90%±4.5% and by 98%±4.3% respectively (both $p<0.0002$).

The uptake of ^{111}In-exendin by BN16 cells (Fig. 2c) was reduced significantly by peptide #3 (42%±6.2%, $p=$ 0.001), peptide #5 (18%±6.4%, $p=0.03$) and most potently by peptide #6 (94%±6.1%, $p<0.0001$), as well as by albumin and Gelo (94%±6.2% and 91%±6.1% respectively, both $p<0.0001$).

The lower doses of 30 μg and 10 μg of the albumin-derived peptides were less effective in inhibiting the uptake of these ^{111}In-labelled peptides (data not shown).

Animal studies

The effect of FRALB-C on the renal retention of ^{111}In-octreotide in rats is summarised in Fig. 3. FRALB-C (1 mg) reduced the renal uptake of ^{111}In-octreotide by 24%±12% ($p=0.02$). Higher doses of FRALB-C (up to 20 mg) did not increase this effect. Lysine (80 mg) and Gelo (20 mg) reduced the uptake by 36%±11% and 27%±9.7%, respectively (both $p<0.003$). FRALB-C had no significant effect on the uptake of ^{111}In-octreotide in the pancreas, adrenals or other organs.

Figure 4 summarises the effect of peptide #6 on the renal retention of ^{111}In-minigastrin, ^{111}In-exendin and ^{111}In-octreotide in rats. Peptide #6 had a pronounced effect on the renal uptake of ^{111}In-minigastrin: 5 mg of peptide #6 reduced the uptake by 88%±9.0% ($p<0.0001$). Gelo reduced the uptake by 77%±8.9% ($p<0.0001$). The renal uptake of ^{111}In-exendin was reduced by 26%±3.4% by peptide #6, whereas Gelo reduced the uptake by 16%± 3.1% (both $p<0.0001$). The highest dose of peptide #6 reduced the uptake of ^{111}In-octreotide by 33%±13% ($p=$ 0.004), comparable to the effect of 20 mg of Gelo (28%± 9.9%, $p=0.002$). The lower doses of 1 mg and 0.2 mg of peptide #6 were less effective in inhibiting the renal uptake of the ^{111}In-labelled peptides.

Peptide #6 significantly reduced the uptake of ^{111}In-octreotide in the adrenals, by 42%±13% ($p=0.0008$), whereas the uptake in the pancreas and other organs was unaffected (Fig. 5). The uptake of ^{111}In-minigastrin and ^{111}In-exendin in organs other than the kidneys was not affected by peptide #6 and no adverse effects of its injection were noted.
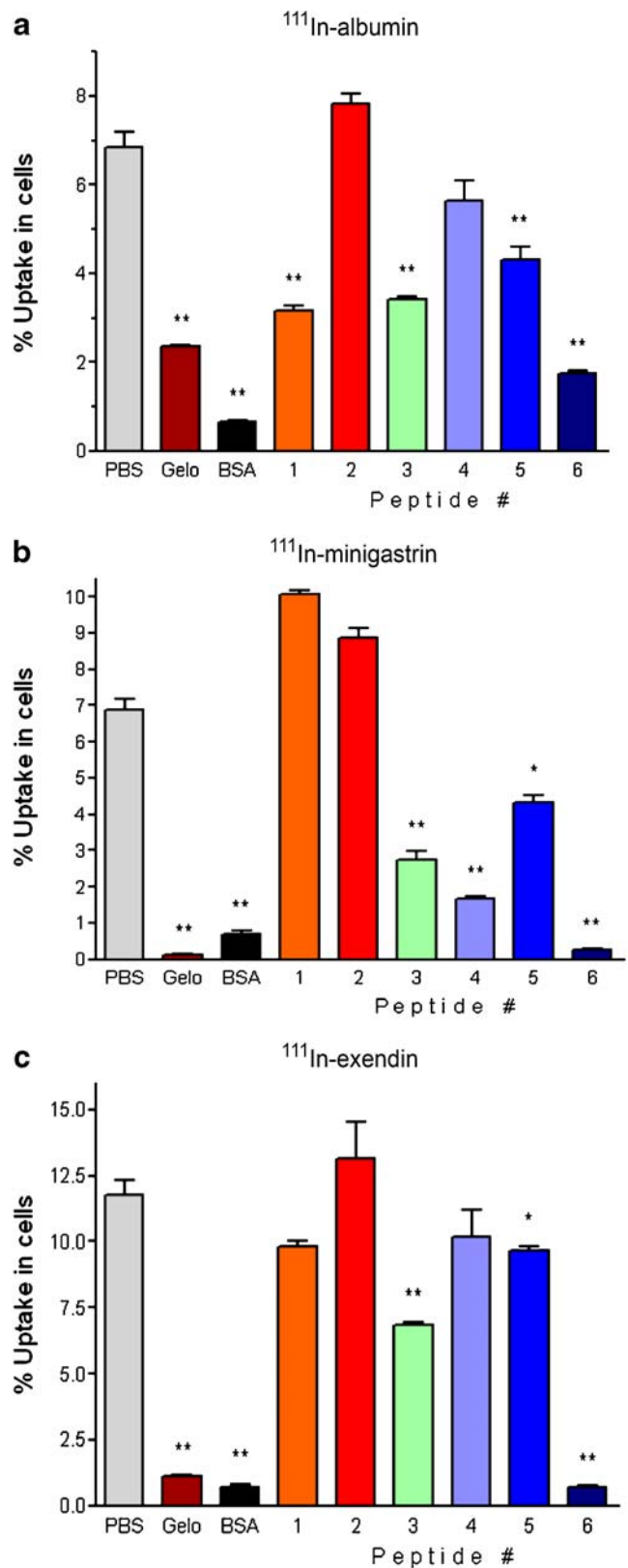
Fig. 2 Inhibition of the uptake of ^{111}In-albumin (a), ^{111}In-minigastrin (b) or ^{111}In-exendin (c) in BN16 cells by intact BSA (10 mg), Gelo (5 mg) or 100 μg of albumin-derived peptides. Results are presented as mean % bound activity; error bars indicate SEM. *$P<0.05$; **$P<0.005$
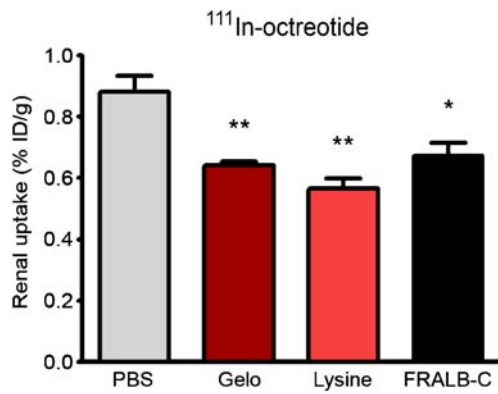
Fig. 3 Kidney activity concentrations 20 h after i.v. injection of ^{111}In-octreotide in rats. One to three minutes prior to the injection of ^{111}In-octreotide, groups of five rats received 0.5 ml of either PBS, lysine (80 mg), Gelo (20 mg) or FRALB-C (1 mg). Results are presented as mean %ID/g, *error bars* indicate SEM. *$P<0.05$; **$P<0.005$

Discussion

Radiation-induced chronic renal damage due to proximal tubular reabsorption and retention of radiolabelled peptides limits the activity dose that can safely be administered in PRRT, thereby limiting the efficacy of this anti-tumour therapy. The renal reabsorption of ^{111}In-octreotide is, at least in part, mediated by megalin [9]. Megalin is a multiligand scavenger receptor that is involved in the renal reuptake of various peptides and proteins, among which albumin [12, 13]. We previously reported that both the protein-based plasma expander Gelo and trypsinised albumin are effective inhibitors of the renal reabsorption of various radiolabelled peptides [1, 21, 22]. In the current study, we aimed to identify albumin-derived peptides that are effective inhibitors of the renal uptake of ^{111}In-labelled minigastrin, exendin and octreotide.

FRALB-C, bovine serum albumin fragmented by cyanogen bromide, proved to be an effective inhibitor of the uptake of ^{111}In-labelled albumin, minigastrin, exendin and octreotide in megalin-expressing cells. In rats, FRALB-C inhibited the renal reabsorption of ^{111}In-octreotide as efficiently as Gelo. The inhibitory effect of FRALB-C was comparable to the effects we previously obtained with trypsinised albumin [1].

FRALB-C is a mixture of at most 28 different albumin fragments. We expected these different fragments to have different affinities for megalin. Therefore, we aimed to further identify specific albumin fragments that inhibited the renal reuptake of radiolabelled peptides efficiently. However, attempts to further purify the FRALB-C mixture by filtration, dialysis and HPLC did not yield sufficiently purified peptides.

Six albumin-derived peptides were screened for their ability to reduce the uptake of ^{111}In-albumin, ^{111}In-exendin and ^{111}In-minigastrin in cells expressing megalin and cubilin. The uptake of ^{111}In-albumin was inhibited by peptides #1, #3, #5 and most potently by #6, whereas peptides # 2 and #4 did not have a significant effect. This suggests that the amino acid sequences of peptides #1, #3, #5 and #6 may be involved in the physiological binding of albumin to its renal receptors. However, since megalin's binding domains facilitate the binding of various endogenous and exogenous peptides, it is also possible that some or all of these sequences are not actually involved in the binding of intact albumin to megalin; for example because they may not be exposed at the surface of the albumin molecule. Each of the peptides #1, #3, #5 and #6 contained both positively and negatively charged amino acid residues, whereas peptides #2 and #4 contained only positive or only negative charges, respectively. It was suggested previously that the distribution of charges rather than the overall charge may be important for ligand binding to megalin
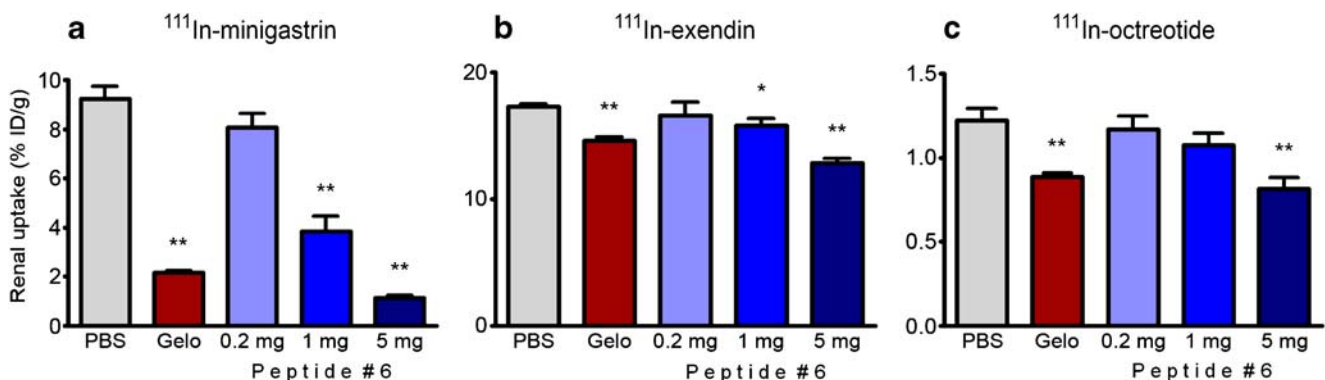

Fig. 4 Kidney activity concentrations 20 h after i.v. injection of ^{111}In-minigastrin (**a**), ^{111}In-exendin (**b**) or ^{111}In-octreotide (**c**) in rats. One to three minutes prior to the injection of the ^{111}In-labelled peptide, groups of five rats received 0.5 ml of either PBS, Gelo (20 mg) or albumin-derived peptide #6. Results are presented as mean %ID/g, *error bars* indicate SEM. *$P<0.05$; **$P<0.005$
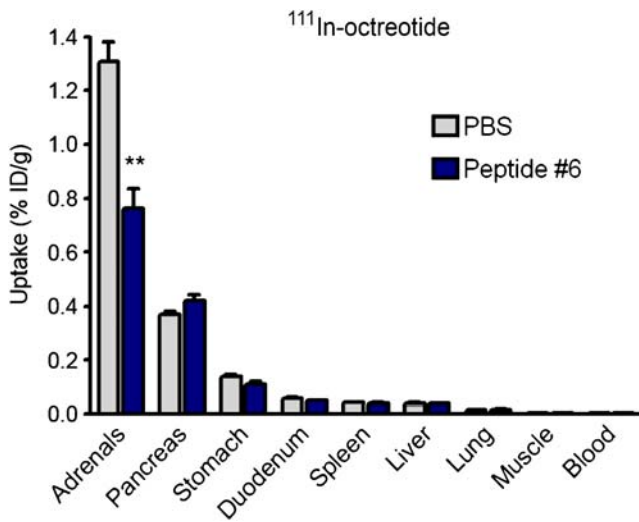
Fig. 5 Biodistribution of ^{111}In-octreotide in rats, 20 h post-injection. One to three minutes prior to the injection of ^{111}In-octreotide, groups of five rats received 0.5 ml of PBS or peptide #6 (5 mg). Results are presented as mean %ID/g; *error bars* indicate SEM. **$P < 0.005$

[13]. Our results suggest that both positive and negative charges might contribute to the binding of albumin to megalin and / or cubilin.

Exendin contains four positively charged and six negatively charged amino acid residues, minigastrin contains seven negatively charged amino acid residues and octreotide contains one positively charged amino acid residue. Similarly to ^{111}In-albumin, the uptake of ^{111}In-exendin by BN16 cells was reduced significantly by peptides #3, #5 and most potently by #6, suggesting that the binding mechanism of ^{111}In-exendin might be similar to that of ^{111}In-albumin. In addition to peptides #3, #5 and #6, peptide #4 significantly reduced the uptake of the exclusively negatively charged ^{111}In-minigastrin as well. Each of the peptides #3–6 contained at least four negatively charged amino acid residues, whereas the peptides that did not reduce the binding of ^{111}In-minigastrin contained three (peptide #1) and 0 (peptide #2) negative charges. This again suggests involvement of minigastrin's multiple negative charges in its renal reuptake. It has been shown previously that the effect of polyglutamic acid chains on the renal reuptake of minigastrin depends on the number of negatively charged residues in these chains [31].

Peptide #6, the larger peptide, inhibited the uptake of ^{111}In-albumin, ^{111}In-exendin and ^{111}In-minigastrin most effectively in vitro, although it was tested at a lower molar concentration than the other peptides. Whether its size, its charge distribution or other properties determine its effectiveness remains to be investigated. This peptide was subsequently studied in vivo.

In rats, peptide #6 reduced the renal uptake of ^{111}In-minigastrin very effectively. The effect of 5 mg of peptide #6

was larger than the effect of 20 mg Gelo. The inhibition of the renal uptake of ^{111}In-exendin by peptide #6 was less pronounced, although its effects on the cell binding of ^{111}In-minigastrin and ^{111}In-exendin were comparable in vitro. A possible explanation for the observed difference is a relatively short plasma half life of peptide #6. Literature suggests longer plasma half lives for exendin than for minigastrin [23, 33]. Peptide #6 might be largely excreted, while exendin is still present in relatively high concentrations in the plasma and the ultrafiltrate. In our previous study [1], trypsinised albumin had more effect on the renal uptake of ^{111}In-exendin than peptide #6 had in this study, which might be explained by a longer half life of this mixture of peptides. Alternative possible explanations for the observed differences between ^{111}In-exendin and ^{111}In-minigastrin in vitro and in vivo include different mechanisms of renal reabsorption (e.g. involvement of different transporters or receptors), a higher affinity of exendin for renal receptors, or general differences in transport mechanisms and kinetics between the kidneys in vivo and the in vitro model.

The inhibition of the renal reabsorption of ^{111}In-exendin and ^{111}In-octreotide by 5 mg of peptide #6 in rats was comparable to the effects of 20 mg Gelo. The effect of FRALB-C on the renal uptake of ^{111}In-octreotide was in the same range. Most other studies using competitive blockers to reduce the renal retention of ^{111}In-octreotide report maximum reductions of around 50% [1, 15, 20–22]. The residual uptake may indicate a contribution of other mechanisms of renal reuptake, such as fluid phase endocytosis [34].

Remarkably, peptide #6 significantly reduced the uptake of ^{111}In-octreotide in the adrenals. Since rat adrenals predominantly express somatostatin receptor subtype 2 (sstr2) [35] and ^{111}In-octreotide has the highest affinity for sstr2 and lower affinities for sstr3 and sstr5 [36], this possibly indicates interference of peptide #6 with the binding of octreotide to sstr2. However, uptake in other organs known to express relatively high levels of sstr2, such as the pancreas and the stomach [37–39], was unaffected. Other mechanisms for the reduced uptake of ^{111}In-octreotide in the adrenals have to be considered and future experiments will have to investigate if specific tumour uptake is affected by peptide #6. Although the peptide is derived from albumin and we did not observe any side-effects in the animal experiments, it is a new substance which may cause toxicity at higher doses. Therefore, the dose of this peptide that can be administered safely has to be determined before clinical use becomes possible.

In summary, peptide #6 is a promising candidate for inhibition of the renal reabsorption of ^{111}In-minigastrin and perhaps of ^{111}In-exendin. To reduce the renal reabsorption

of radiolabelled peptides more efficiently, a combination of two or more competitive inhibitors of endocytosis and other approaches may prove to be useful.

Conclusion

Albumin digested by CNBr (FRALB-C) efficiently reduced the uptake of ^{111}In-labelled albumin, octreotide, minigastrin and exendin in megalin expressing cells, and reduced the renal retention of ^{111}In-octreotide in rats. In vitro, the effect of albumin-derived peptide #6 on the uptake of ^{111}In-exendin and ^{111}In-minigastrin was comparable to the effect of FRALB-C and Gelofusine. In rats, peptide #6 efficiently reduced the renal retention of ^{111}In-labelled octreotide, exendin and especially minigastrin, opening up new possibilities for the reduction of kidney damage in peptide receptor radionuclide therapy with less chance of side-effects.

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