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TECHNICAL NOTE

Quantification of renal low-molecular-weight protein handling in the intact rat

MARIJKE HAAS, DICK DE ZEEUW, ANNIE VAN ZANTEN, and DIRK K.F. MEIJER

Department of Internal Medicine, Division of Nephrology, and Department of Nuclear Medicine, University Hospital; and University Centre for Pharmacy, Department of Pharmacology and Pharmacotherapy, Groningen, The Netherlands

In the kidney, low-molecular-weight proteins (LMWPs) are relatively freely filtered and after reabsorption by the proximal tubular cells, intracellularly catabolized into small peptides and single amino acids [1]. As such, the kidney plays an important role in the elimination of circulating LMWPs. Studies aimed at quantifying the renal contribution to catabolism of LMWPs revealed rather variable results. On the one hand a major, if not exclusive, renal contribution (60 to 90%) to the total clearance was claimed on the basis of the renal extraction measurements of the particular LMWP [2–4]. On the other hand, several studies showed a considerable lower renal contribution (30 to 60%), calculated on the basis of the actual amount of administered LMWP measured in the kidneys [6–10]. The question is whether methodological differences can explain the observed discrepancies. With regard to the renal rate of degradation, large differences have been found for individual LMWPs. Previous studies on this topic were performed either *in vitro* kidney preparations [2, 3, 7, 11], or by measuring the renal LMWP concentration in extirpated kidneys at different time intervals [7, 8]. The disadvantage of the latter *in vivo* studies is the low time resolution. Bianchi et al introduced an elegant non-invasive technique with a high time resolution with which an indication of the renal degradation of a LMWP was obtained [9].

In the present study we studied the renal handling of the three LMWPs cytochrome-c (Cy), lysozyme (Ly) and aprotinin (Ap), chosen on the basis of their alleged difference in renal half life [9]. We used a technique based on that previously reported by Bianchi et al in which the renal handling of a radiolabeled LMWP was monitored by external counting in the intact rat [9]. We modified this technique to enable quantification of both renal uptake and degradation of a ^{123}I-labeled LMWP. To validate the renal uptake data obtained with the ^{123}I-LMWP, we used a second label, ^{131}I-tyramine-cellobiose (TC), a radiolabel that is retained within the cell in which it is internalized.

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Methods

Materials

Cytochrome-c (horse heart; molecular wt 12,400; pI 10.6), lysozyme (egg white; molecular wt 14,300; pI 11) and aprotinin (bovine lung; molecular wt 6,500; pI 10.5) were obtained from Sigma (Axel, The Netherlands).

Experimental set-up

One week before the experiments, female Wistar rats were instrumented with a permanent venous heart cannula [12]. The day before the experiment, the animals were fasted overnight and received 2 mg sodium-iodine intravenously to minimize the accumulation of unbound iodine in the thyroid glands and stomach [13]. Anesthesia was induced with halothane and a single dose of 30 mg/kg pentobarbital. During the experiment, anesthesia was maintained by continuous infusion of 4 mg/kg/hr pentobarbital. In addition, the rats received a continuous infusion of 5% glucose (2.0 ml/hr) to induce a stable urine production and 6 mg/hr 3-monoiodo-l-tyrosine (MIT) to saturate the deiodinases [14]. Three rats were properly fixed on a middle-energy collimator of a gamma camera. A window was selected on the peak energy of ^{123}I and ^{131}I with a width of 150 and 250 keV, respectively. Body temperature was monitored and maintained on 38°C with a heating pad and a lamp. Urine was collected continuously via a short cannula in the urinary bladder, draining into a tube situated on the collimator. The urine tube was exchanged after a half or one hour collection time. After a stabilization of one hour, 1.1 MBq ^{123}I-LMWP was co-injected with 0.55 MBq ^{131}I-TC-LMWP intravenously. The gamma camera recorded the activity of both iodine isotopes in one or five minute time frames for three to eight hours, depending on the LMWP studied. The radioactivity time course of the right kidney, urine and total body was subsequently plotted after analysis of the respective "regions of interest." The amount of radioactive iodine within the total body in the first five minutes after injection was assumed to be 100% of the injected dose. Blood and urine samples were taken to measure the ratio of protein bound, amino acid bound or unbound radioactivity. For each studied LMWP six rats were analyzed.

^{123}I-LMWP synthesis

^{123}I-LMWP labeling was performed according to the chloramine-T method of Hunter and Greenwood [15]. In short, 50 μl

protein (10 mg/ml) and 10 μl chloramine-T (5 mg/ml) were added to 37 MBq $Na^{123}I$ in 100 μl phosphate buffer (0.2 M, pH 7.4) and mixed for 60 seconds. The reaction was stopped by addition of 25 μl metabisulfite (2.5 mg/ml). Free iodine was removed by a separation on a Sephadex G25 column. The protein fraction was used within two hours. Just before administration, 95% (Cy), 95% (Ly) and 94% (Ap) of the iodine were protein bound.

^{131}I-TC-LMWP

Synthesis of tyramine-cellobiose (TC) was performed by the reductive amination of cellobiose with tyramine [16, 17]. In short, 10 mmol cellobiose, 10 mmol tyramine hydrochloride and 10 mmol propionic acid were dissolved in 40 ml methanol. Sodium cyanoborohydride (12 mmol) was dissolved in 15 ml methanol and added drop-wise. The mixture was refluxed overnight. Acetone (300 ml) was added and the precipitate was filtered. The precipitate was dissolved in water overnight and applied to a cation exchange column (Dowex W50-X4; size 0.25 × 20 cm). The column was eluted with 0.5 M ammonia and the absorbance measured at 279 nm. The first peak was collected and lyophilized twice in order to remove traces of ammonia. The yield was about 40%.

^{131}I-TC labeling and subsequently coupling to LMWP was also carried out according to the method of Hysing and Tolleshaug [16], the modified version of Pittman et al [17]. In short, in an iodogen-coated tube, 10 μl tyramine-cellobiose (TC) (0.01 M) in phosphate buffer (0.02 M, pH 7.2) and 92 MBq $Na^{131}I$ were incubated for 30 minutes at room temperature. The reaction was stopped by the addition of 10 μl sodiumsulfite (0.05 M) and 5 μl potassium-iodine (0.1 M). The solution was transferred to a clean tube to couple the iodinated TC to the LMWP. Cyanuric chloride (30 nmol) in 20 μl acetone and 5 μl NaOH (0.01 M) were added. After mixing for 30 seconds, 10 μl LMWP (100 mg/ml) in carbonate buffer (0.01 M, pH 9) was added and the ^{131}I-TC-LMWP complex was separated from free compounds on a Sephadex G25 column. The protein fraction appeared to be very stable and was used within 40 hours. Just before administration, 100% (Cy), 100% (Ly) and 99% (Ap) of the iodine was protein bound.

Blood and urine analysis

The nature of the radioactive compounds in blood and urine samples was determined according to the method of Albert and Raymond Keating [18], slightly modified for analyzing small samples. In short, the LMWP coupled radiolabel in urine was measured by a trichloroacetic acid (TCA) precipitation of the LMWP. Urine (100 μl) and 400 μl 0.5% bovine serum albumin (BSA) were added to 250 μl TCA (15% TCA, 0.1% NaI, 0.1% tyrosine). After a high speed centrifugation of one minute the precipitate was washed with 200 μl 1 M NaOH, to remove co-precipitated free iodine, and reprecipitated with 300 μl TCA. After centrifugation, the radioactivity in the precipitate represented the LMWP coupled iodine. The TCA soluble radioactivity was subsequently analyzed for amino acid coupled and free iodine. The TCA supernatant was diluted with ±50 μl 32% NaOH and Congo red, to change the solution to a basic environment. Addition of 10 μl 50% HNO_3 and subsequently 250 μl 10% $AgNO_3$ in 2% HNO_3, was performed for the crystallization of the free iodine with silver. After centrifugation, the crystals were discarded to prevent interferences with the counting of radioactivity. The radioactivity in the supernatant represents the amino acid bound iodine. The unbound iodine could be calculated from the difference between radioactivity in the untreated urine sample and the protein plus amino acid fractions. The procedure for plasma samples was the same except for the first step: 10 μl plasma and 400 μl 0.35% BSA were added to 250 μl TCA. Radiolabeled aprotinin appeared to be TCA-precipitable for only 50%. The rest was recovered in the unbound iodine fraction after AgI crystallization. The administered cytochrome-c and lysozyme were analyzed likewise. The protein fraction of the administered aprotinin was analyzed by thin layer chromatography.

Quantification of the gamma camera data

The data on radioactivity of the ^{123}I and ^{131}I measured over the different regions in the rat by external counting had to be transformed to the actual amount of label within the organ. To enable this, we studied the following biasing parameters: (1) the degree of scattering of the gamma emittance by surrounding tissue, (2) the spillover of ^{131}I pulses into the ^{123}I channel, and (3) the background. The relative contribution of these factors was measured in a phantom rat model with the dimensions similar to the *in vivo* situation. A plastic tank (19 × 9 × 2 cm^3), representing the total body of the rat, held two artificial plastic kidneys (1.3 cm^3 × 0.5 cm) and a urine tube of 1 ml, the average volume present in the *in vivo* experiment. The different compartments were filled with saline and various concentrations of ^{123}I or ^{131}I. Different sizes of the regions of interest were analyzed for the iodine radioactivity. This resulted in the following corrections for the *in vivo* experiments. For the ^{123}I, the region of the total body and urine tube contained the same amount of radioactivity compared to the total field of the camera. To estimate the total amount of ^{123}I-radioactivity present in the right kidney, a region of interest was needed with a size twice as large as the actual kidney. No corrections were needed for the background or the scattering effect of the radioactivity present in the left kidney. The counting efficiency of the ^{131}I appeared to be much lower. The total body and urine tube region contained 80%, whereas the 2 cm^2 sized right kidney region only comprised 47% of the total field radioactivity. Furthermore, a correction was made for the spillover of ^{131}I pulses into the ^{123}I channel. This spillover appeared to be 35% for the kidney and 50% for the total body and urine tube. No corrections were needed for background or scattering. To ensure that indeed no background corrections were needed, we studied the effect of an estimated background on the renal time-activity curve of ^{123}I-cytochrome-c (the LMWP that will be mostly effected). This process did not have a major impact on the results since the rates of degradation of the ^{123}I-cytochrome-c were not changed significantly. To validate whether the corrections of the ^{131}I gamma camera data were justified in the *in vivo* studies with the combination of both radiolabels, we compared the renal uptake of ^{131}I-TC-lysozyme and ^{123}I-TC-lysozyme in two separate *in vivo* experiments. The renal uptake appeared to be similar which proves that the ^{131}I corrections estimated from the phantom model experiment are indeed valid for the *in vivo* situation.

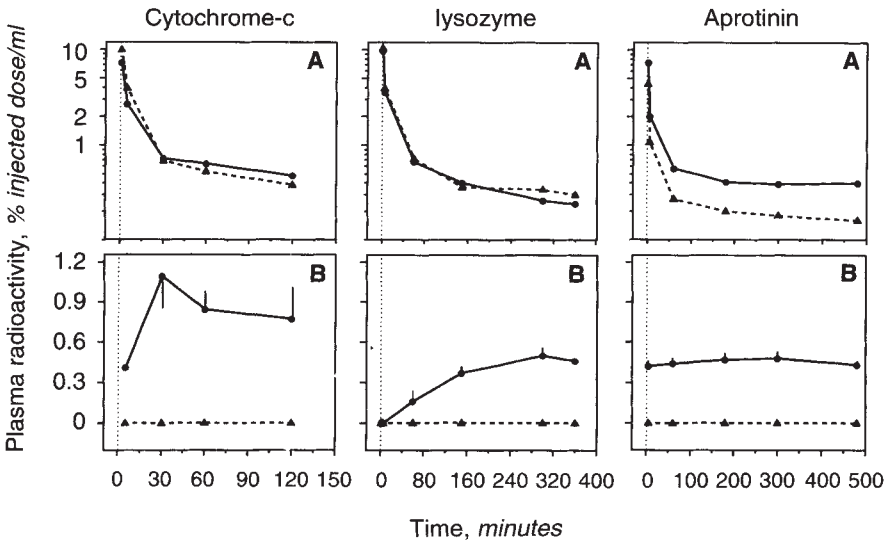

Fig. 1. *Plasma radioactivity as a function of time after the intravenous injection of ^{123}I and ^{131}I-TC radiolabeled cytochrome-c* **(left panels)**, *lysozyme* **(middle panels)** *and aprotinin* **(right panels)** *in the rat.* **Panels A.** Plasma disappearance of ^{123}I-LMWPs (solid line) and ^{131}I-TC-LMWPs (dotted line), measured as TCA precipitable radioactivity. **Panels B.** Plasma appearance of the ^{123}I-degradation products (solid line) and ^{131}I-TC-degradation products (dotted line) as the TCA soluble radioactivity. Values are given as mean + SEM [Note: the SEM of the plasma concentration LMWP (panels A) is not visible since the SEM falls within the size of the marker].

Pharmacokinetic and statistical analysis

The pharmacokinetic analysis of the plasma and kidney time-activity curves was performed using a computer program for non-linear curve-fitting, MultiFit (Department of Pharmacology and Therapeutics, University of Groningen), using the simplex algorithm [19]. Initial parameter estimates are automatically obtained by a curve-stripping procedure. The reciprocal of the y-value predicted by the model was used as the weighing factor [20].

All data are expressed as mean ± SEM. Statistical analysis was performed with the Wilcoxon rank test.

Results

Plasma disappearance of the radiolabeled LMWPs

After the intravenous injection of the ^{123}I and ^{131}I-TC radiolabeled LMWPs cytochrome-c, lysozyme and aprotinin, the plasma disappearance was determined by measuring the TCA precipitable plasma radioactivity in time (Fig. 1A). The results show that for each LMWP a similar biphasic plasma disappearance pattern was found for the two labels, ^{123}I and ^{131}I-TC (Table 1). Apparently, the difference in the two labels did not affect the plasma elimination of the LMWP. Interestingly, compared to one another, the distribution volume, estimated from the second phase of the plasma disappearance, is markedly different: 11 ml for cytochrome-c, 20 ml for lysozyme, and 50 ml for aprotinin.

Renal ^{123}I time-course

The body distribution of the radiolabel ^{123}I was monitored continuously by external counting. Following the intravenous injection, a large amount of the ^{123}I-LMWP is directly taken up by the kidney. After this first pass uptake, the renal uptake continues gradually in combination with a degradation of the LMWP in the kidney. Kinetic data of renal uptake and degradation were obtained by computer-aided kinetic analysis. Figure 2 shows the renal time-course of the three ^{123}I radiolabeled LMWPs: cytochrome-c, lysozyme and aprotinin. Furthermore, the regression lines are drawn which represent the renal rate of uptake (a) and degradation (b). The results (Table 2) show that the rate of renal uptake as well as degradation is very different for the three LMWPs. Lysozyme is taken up and degraded twice as fast as aprotinin. Cytochrome-c's rate of uptake is eight times higher while the majority degrades 40 times faster than aprotinin. To verify that ^{123}I degradation products were not retained within the kidney, thus obscuring our degradation analyses, we measured the amount of ^{123}I bound to lysozyme in extirpated kidneys. At the end of the experiment, 84 ± 2% (N = 3) of the ^{123}I in the kidney appeared to be bound to lysozyme. This is in agreement with other studies [1, 6] which show that at least 85% of renal radioactivity is protein bound, independent of the analysis time after injection.

Table 1. Plasma disappearance of radiolabeled LMWPs after intravenous injection in the rat

LMWP	Phase (%)	Plasma half-life *min* ^{123}I-LMWP	^{131}I-TC-LMWP	
Cytochrome-c	1st (94)	2.1, 2.4	2.4, 2.8	(N = 2)
	2nd (6)	133, 167	90, 127	
Lysozyme	1st (94)	2.6 ± 0.3	3.0 ± 0.2	(N = 4)
	2nd (6)	180 ± 23	259 ± 17	
Aprotinin	1st (96)	2.0 ± 0.2	1.9 ± 0.2	(N = 5)
	2nd (4)	657 ± 62	646 ± 126	

Data are given as mean ± SEM, except for cytochrome-c where the results of both experiments are separately given. The values in parenthesis denote the contribution of the two phases relative to the total plasma disappearance. The first phase represents the distribution and first pass elimination of the LMWP from the plasma, the second phase the plasma elimination.

Renal ^{131}I time-course

The renal time-course of the ^{131}I-TC-LMWPs was also recorded by external counting. After the first pass uptake, the renal uptake continues gradually until a plateau level is reached (Fig. 2). This plateau level very likely represents the total amount of the LMWP that accumulates in the kidney, since the ^{131}I-TC label remains trapped in the tubular cell. The rate of

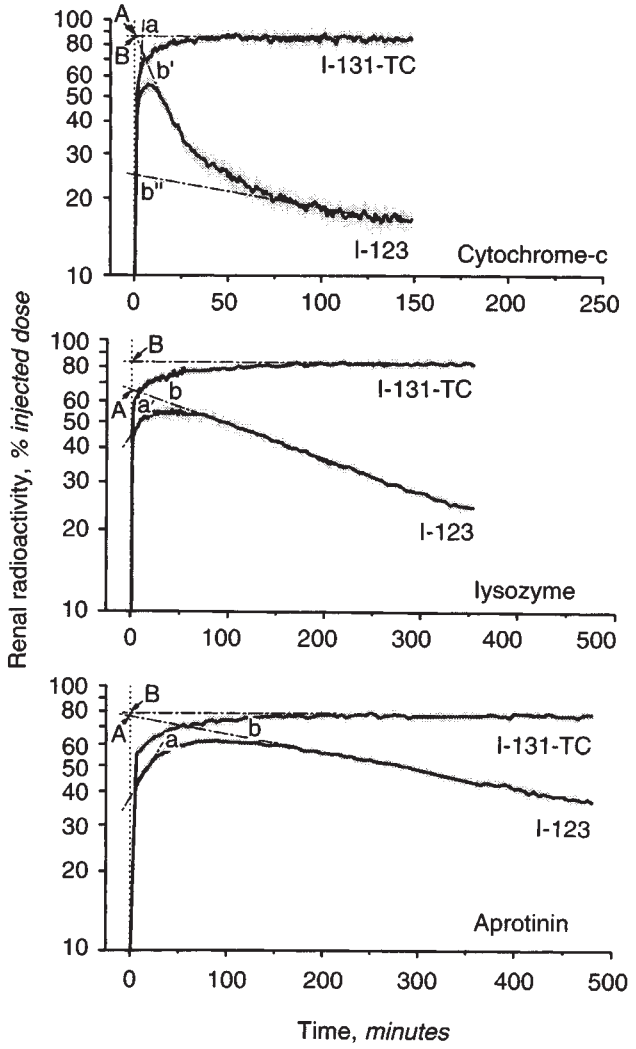

Fig. 2. *Renal time course of ^{123}I and ^{131}I-TC radiolabeled cytochrome-c (upper panel), lysozyme (middle panel) and aprotinin (lower panel) after intravenous injection in 6 rats.* Values are given as mean ± SEM (shaded area). Regression lines (obtained by kinetic analyses) are drawn, representing the rate of renal uptake (a) and renal degradation (b) of the ^{123}I-LMWP. The degradation of cytochrome-c appeared to be biphasic (b′ and b″). Total amount of renal uptake is presented by the extrapolated degradation curve of ^{123}I-LMWP to the time of injection (A) and by the maximum level of radioactivity of ^{131}I-TC-LMWP (B).

uptake appears to be similar to the uptake kinetics of ^{123}I-LMWP. This indicates that the difference in the two labels does not affect the renal uptake of the LMWP.

Total amount of renal LMWP

The total amount of renal uptake was obtained in two different ways: first of all, by extrapolating the renal degradation curve of ^{123}I-LMWP to the time of injection. This is visualized in Figure 2 by the percentage of injected dose at time zero on the regression line of degradation (A). Secondly, it was found by estimating the renal accumulation of ^{131}I-TC-LMWP at the plateau level (Fig. 2, visualized by the horizontal line to time zero: B). The results are listed in Table 3. The data show that the two methods are in good agreement for the proteins,

Table 2. Renal kinetics of ^{123}I-LMWP after intravenous injection in 6 rats

LMWP	Renal half-life $t_{1/2}$ *in min*		
	Uptake		Degradation
Cytochrome-c	4.0 ± 0.1	1st	11 ± 1 (83 ± 6%)
		2nd	222 ± 46 (17 ± 1%)
Lysozyme	14.2 ± 1.3		252 ± 26 (100%)
Aprotinin	31.2 ± 0.9		443 ± 28 (100%)

Data are given as mean ± SEM. The $t_{1/2}$ uptake is defined as the time in which the renal content, after the first pass uptake, increases with 50%. The $t_{1/2}$ degradation is the time in which 50% is released from the kidney. Numbers in parenthesis denote the contribution of the different degradation phases relative to the total renal degradation of the LMWP.

Table 3. Total amount of renal uptake (% of injected dose) of radiolabeled LMWPs after intravenous injection in 6 rat

LMWP	Renal uptake (%)	
	^{123}I-LMWP	^{131}I-TC-LMWP
Cytochrome-c	89 ± 4 (72–108)	88 ± 5 (67–99)
Lysozyme	67 ± 6 (49–84)	84 ± 3 (77–98)
Aprotinin	78 ± 2 (69–84)	79 ± 3 (70–90)

Data are given as mean ± SEM (values in parenthesis denote the range). Data are obtained by kinetic analysis of the renal time course of ^{123}I and by estimating the stable maximum level of the renal ^{131}I radioactivity.

cytochrome-c and aprotinin. For lysozyme, the ^{123}I uptake data are lower ($P = 0.05$) and exhibit more variation (49 to 84%) compared to the ^{131}I-TC results (77 to 98%). Striking is the high and remarkably similar renal accumulation of the three LMWPs: 88, 84, 79% of the injected dose for ^{131}I-TC labeled cytochrome-c, lysozyme and aprotinin, respectively.

Degradation products

Plasma samples were analyzed for TCA-soluble radioactivity, representing the degradation products of the intravenous injected radiolabeled LMWP. No detectable amounts of ^{131}I degradation products were found in the plasma, suggesting that the ^{131}I-TC label is indeed trapped in the cell in which it is internalized. This is in contrast to the ^{123}I degradation products, of which the plasma appearance pattern (Fig. 1B) seems to be in agreement with the rate of LMWP degradation in the kidney (Fig. 2). The urine accumulation of radioactivity was monitored continuously by external counting. Subsequently, urine samples were analyzed for protein, amino acid bound and unbound radioactivity by TCA precipitation and silver crystallization. As in plasma, no detectable amounts of ^{131}I-TC-degradation products were found in the urine. Figure 3 shows the accumulation of the ^{123}I degradation products in the urine. Since the kidney is the major site of LMWP degradation, supposedly the ^{123}I degradation products in the urine are mainly originating from the kidney. Indeed, the appearance of the ^{123}I degradation products in the urine appears to coincide with the renal disappearance of ^{123}I (Fig. 2). During the time of the study with cytochrome-c, lysozyme and aprotinin, 73 ± 3%, 61 ± 2% and 43 ± 3% of the injected ^{123}I were released from the kidney, respectively. In the same period 44 ± 3% (Cy), 40 ± 4% (Ly) and 25 ± 2% (Ap) of the injected dose of ^{123}I were recovered as

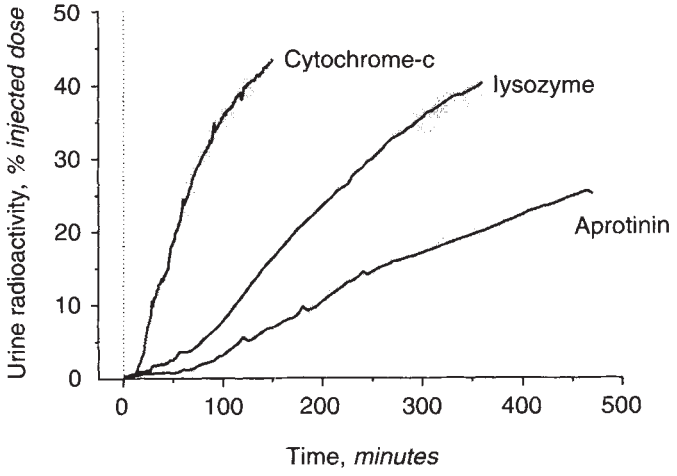

Fig. 3. *Time course of urinary excretion of ^{123}I after intravenous injection of ^{123}I radiolabeled cytochrome-c, lysozyme and aprotinin in 6 rats.* Values are given as mean ± SEM. More than 95% of the ^{123}I appeared to be TCA soluble, representing radiolabeled degradation products. No ^{131}I was recovered in the urine.

degradation products in the urine. This implies that 60% (Cy), 66% (Ly) and 59% (Ap) of the degradation products released from the kidney during the time of the study were recovered in the urine. This may be due to the large distribution volume of the ^{123}I-breakdown products in the body. From these degradation products 67 ± 5% (Cy), 73 ± 5% (Ly) and 91 ± 1% (Ap) were bound to amino acids. The rest was free ^{123}I. This indicates that the efficiency of deiodinase inhibition by the MIT was not complete and was different for the three LMWPs studied. No detectable amounts of LMWP bound iodine were found in the urine, suggesting that the injected tracer amounts of LMWPs were maximally reabsorbed by the proximal tubular cell.

Discussion

In the present study, we show that we can quantify both renal uptake and degradation of a ^{123}I-radiolabeled LMWP in an intact rat by registering the renal time-activity curve with a gamma-camera and applying subsequent kinetic analysis to the obtained curve.

The total amount of renal uptake was approximately 80% of the administered dose for all the three LMWPs, cytochrome-c, lysozyme and aprotinin. An accumulation up to 80% of the injected dose is in good agreement with some of the studies in the literature. First of all, our results are in line with the renal extraction studies, which show 73% extraction for parathyroid hormone [2], 67% for growth hormone [3] in rats, and 90% for growth hormone in sheep [4]. However, such studies may show considerable variability since they are based on a combination of *in vivo* and kidney perfusion data [2, 3] or on measurements of minute differences in protein concentrations in renal arterial and venous blood [4]. Secondly, our uptake data match the 90% contribution of the kidney in the catabolism of lambda-1-chain reported by Waldmann, Strober and Mogielnicki [5]. These authors calculated this value using the metabolic rate of the LMWP and the creatinine clearance in healthy individuals.

A more direct way to study the renal handling of LMWPs is the measurement of the actual amount of LMWP in the kidney. In spite of the similar experimental approach, most of such studies suggest a markedly lower renal uptake and differences among the various LMWPs compared to the 80% for all the three LMWPs in the present study. What are the possible explanations for these differences? Just, Christensen, Ottensen and Bianchi et al [6–9] injected iodinated LMWPs intravenously in the rat and measured the radioactivity in extirpated kidneys after different time intervals. Depending on both the LMWP and on the time point with the highest radioactivity, 29 to 58% of the injected dose was maximally recovered in the kidneys. However, this renal radioactivity is likely to be an underestimation of the total amount of LMWP uptake. First of all, it is difficult to determine the exact time point on which the renal amount is maximal. Bianchi et al solved this by establishing the maximum beforehand by continuous external counting [9]. Secondly, the level of this "maximum" is determined by both uptake and degradation of the LMWP in the kidney. In our study with ^{123}I-LMWP we bypassed these limitations, since our kinetic analysis of uptake is based on correction for the renal rate of degradation of the ^{123}I-LMWP.

To validate the calculated amount of renal uptake of ^{123}I-LMWP, we co-injected ^{131}I-TC-LMWP. Since the ^{131}I-TC label remains trapped in the kidney cell, there will be no interference with the renal degradation of the LMWP. This method was used before by Hysing, Tolleshaug and Curthoys [10]. Compared to us, they found the rather low renal uptake values of 43% for cytochrome-c and 35% for lysozyme. Although we used the same procedures of iodination and coupling, we did not detect any radioactivity in the liver. This in contrast to their study in which a hepatic accumulation of 19% of the injected dose was found. Such an hepatic uptake may indicate denaturation or aggregation of the protein. Besides this, other factors should be considered like the anesthesia and the computation of the amount of radioactivity in the extirpated kidney relative to the administered dose. In our study, the reliability of the ^{131}I-TC-LMWP results largely depend on the proper corrections of the gamma camera data. However, evidence that these corrections did not significantly effect our uptake data is shown by the very similar data of the separate study with ^{123}I-TC-lysozyme in which no corrections were needed.

Apart from the total amount of renal uptake, we quantified the rate of renal uptake and degradation of the LMWP. There is a clear difference in the rate of renal uptake between the three LMWPs. Differences in renal extraction appear not to explain this phenomenon, since the renal clearance of the three LMWPs are reported to be very similar and close to the glomerular filtration rate [1]. In this study, the renal clearance, calculated from the renal rate of uptake and the distribution volume, appeared to be about 1.9, 1.0 and 1.1 ml/min for cytochrome-c, lysozyme and aprotinin, respectively. In agreement with the literature, these data are indeed quite comparable to one another and close to the glomerular filtration rate, which makes it rather unlikely that the difference in renal extraction explains the difference in rate of renal uptake. This leaves open the option of a difference in extra-renal distribution volume between the different LMWPs. Evidence for this option is clearly shown by the difference in estimated distribution volume of the three LMWPs.

The renal rate of degradation of the three LMWPs, cytochrome-c, lysozyme and aprotinin have not been quantified before. The estimated data from the literature are in line with our results. The rate of renal degradation of cytochrome-c is high (within minutes) [7, 9, 11], of lysozyme intermediate [8, 9], and of aprotinin low (several hours) [9].

In conclusion, we show that the renal handling of LMWPs can be quantified non-invasively in the intact rat by applying kinetic analysis on the renal time-activity curve of ^{123}I-labeled LMWP, obtained with a gamma camera.

For the three tested LMWPs, cytochrome-c, lysozyme and aprotinin the total amount of renal uptake is high (about 80%) and strikingly similar, whereas their renal degradation rate is quite different. The presented technique may not only be used to extend our knowledge in renal LMWP handling in animal experimental settings, but may also be of use in a clinical environment.

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Reprint requests to Dr. D. de Zeeuw, M.D., Department of Internal Medicine, University Hospital, Oostersingel 59, NL-9713 EZ Groningen, The Netherlands.

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