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PROTEINS, GROWTH FACTORS, AND PROGRESSION OF KIDNEY DISEASE

UNINEPHRECTOMY INCREASES KIDNEY β2-MICROGLOBULIN: CAN IT PLAY A ROLE IN THE PROGRESSION OF KIDNEY DAMAGE?

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ABSTRACT

β2-microglobulin (β2M) is highly accumulated by the kidneys of normal rats. The aim of this study was to verify if uninephrectomy can modify the renal uptake of labeled β2M. For this purpose the radioactivity of plasma and those of the remaining kidney, liver and urine have been measured in uninephrectomized rats (NX) and in controls (C) at different times after the injection as i.v. bolus of ^{131}I-β2M. The experiments were performed in 114 Sprague-Dawley male rats. Fifty seven animals underwent right nephrectomy, the other animals being the C. NX and their C were divided in 3 groups, studied 2, 4 and 6 weeks after nephrectomy, respectively. Part of the animals were sacrificed 12 min after the injection of labeled β2M (peak-time, i.e. time of highest kidney accumulation of ^{131}I-β2M in the normal rat) and part 10 min later.

The results demonstrate that:

– uninephrectomy increases plasma retention of ^{131}I-β2M
– kidney uptake (total and per gram) is always higher in NX

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– liver uptake (much lower than that of kidney) is not influenced by uninephrectomy
– urine excretion of radioactivity is minimal in both NX and C.

The behavior of β2M is similar to that we previously observed with α1-microglobulin and lysozyme. The higher kidney content of some low mw proteins after uninephrectomy could play a role in the progressive reduction of renal function determined by the reduction of renal mass.

Key Words: Uninephrectomy; β2-Microglobulin; Hypermetabolism; Progression of kidney damage

INTRODUCTION

Beta2-microglobulin (β2M) is an anionic low mw protein (mw 11,800 pI 5.8) of great interest in nephrology for its amyloidogenic property in long-term dialysis patients (1–5). In the past years the important role of the kidney in the extraction and metabolism of β2M has been elucidated in rat, in dog and in humans (6–9). Like other low mw proteins, β2M is filtered by the glomeruli (glomerular sieving coefficient 0.94) and almost completely reabsorbed by the proximal tubular cells, where it is metabolized (10). Most probably, β2M is removed from the blood only by the kidney and its plasma concentration increases with the decreasing of renal function (11–13). Previously we studied in normal rats, body distribution, renal kinetics and kidney uptake of radioiodinated β2M (7).

The aim of the present study is to establish if the reduction of renal mass can modify the renal handling of β2M. For this purpose the renal kinetics of ^{131}I-β2M was investigated in uninephrectomized rats (NX) and in controls (C) determining plasma level, kidney content, liver content and urine excretion of ^{131}I-β2M at different intervals after surgery.

In this study human β2M was employed. It has been assumed that rat kidney cannot distinguish human from rat β2M in an acute experimental situation.

METHODS

β2-Microglobulin

β2M (mw 11, 800, pI 5.8) was obtained from the urine of patients with tubular proteinuria and purified by gel filtration and ion-exchange chromatography (Dakopatts, Denmark). It was iodinated with ^{131}I by the chloramine t method (14). The iodination efficiency was approximately 80% and the specific activity was about 20 mCi/mg protein. Radioiodinated β2M was employed within 5 days from the labeling. Prior to each experiment,

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free iodine was removed by ion-exchange chromatography. After this purification, free iodine was less than 1%.

Animals

Sprague-Dawley male rats were employed (n 114, body weight 220–490 g, mean 327 ± 60 SD). They were allowed free access to tap water and standard rat chow containing 15% of proteins (MIL Morini, Reggio Emilia, Italy).

Experimental Design

Experiments were performed under anesthesia induced by intraperitoneal injection of penthotal sodium (Abbott, Roma, Italy), 50 mg/Kg body weight in 5% aqueous solution. Fifty-seven rats were submitted to right nephrectomy, an equal number being the C. Nephrectomy was performed by laparotomy, ligation of the right renal pedicle and removal of the right kidney. NX were divided into three groups, studied 2, 4 and 6 weeks after nephrectomy, respectively, together with an equal number of C. ^{131}I-β2M was injected as a bolus into a tail vein at a dosage of approximately 25 μCi corresponding to 1 μg of protein. The results of a previous study indicate that in the rat the time of highest kidney radioactivity (peak-time) after bolus injection of ^{131}I-β2M is about 12 min as shown in Figure 1 (7). Part of the animals (NX and C) of each group were sacrificed at the kidney peak-time (12 min after the injection of ^{131}I-β2M) and the remainder 10 min later (that is

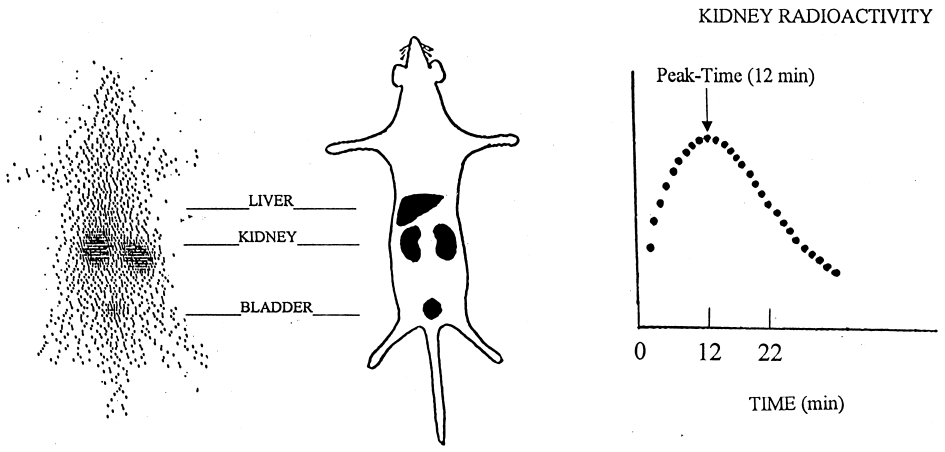

Figure 1. Total body scan (left) and time-course curve of kidney radioactivity (right) recorded in a normal rat after i.v. injection of ^{131}I-β2-microglobulin.

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at the 22nd min after the injection), when kidney radioactivity is decreasing. Sacrifice was performed by opening the chest and rapidly drawing 7–10 mL of blood from the heart. Immediately after sacrifice, left kidney, liver and total urine were removed. Plasma, left kidney and liver were weighed using a precision balance (Cobos 704, Spain). The radioactivity of the injected dose and those of plasma, kidney, liver and urine were measured using the same scintillation counter (Italelettronica, Italy).

The results are expressed as percent of the injected dose. All the results are presented as mean ± SD. Data were analyzed using Student's unpaired t test. Differences are considered significant if the p value was less than 0.05.

RESULTS

Plasma (1g) – After injection of ^{131}I-β2M plasma radioactivity is higher in NX than in C. Such an increase is particularly evident at the second and fourth week after nephrectomy (peak-time) and at the second week (10 min after peak-time) (Figure 2).

Kidney (total and 1g) – Total kidney uptake of ^{131}I-β2M is markedly higher in NX than in C at any time after nephrectomy, either at the peak-time

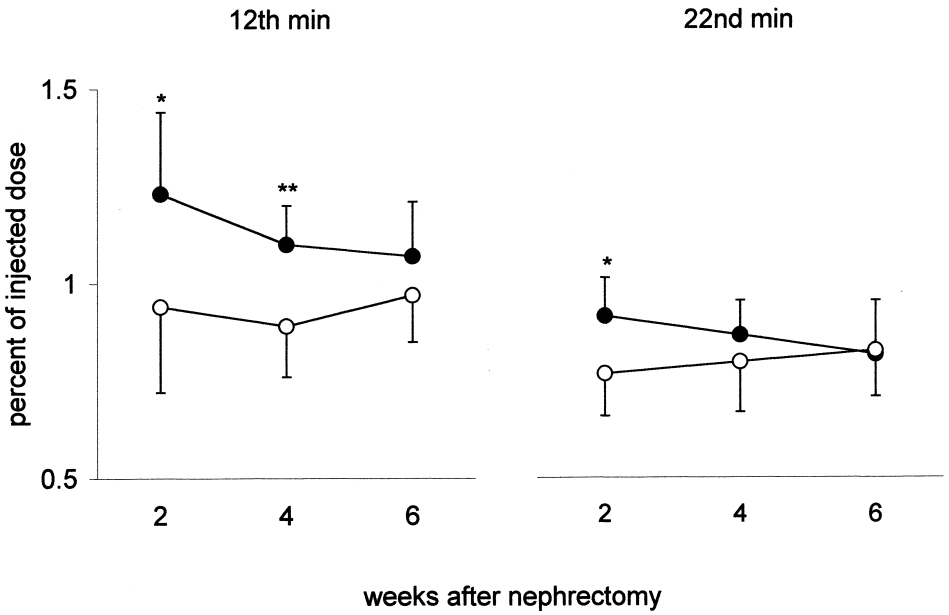

Figure 2. Plasma radioactivity of ^{131}I-β2-microglobulin (radioactivity per gram of plasma, percent of the injected dose) in uninephrectomized rats (●) and in controls (○) 2, 4 and 6 weeks after nephrectomy. On the left the values at the 12th min after i.v. injection of ^{131}I-β2-microglobulin (peak-time); on the right those at the 22nd min. Mean values ± SD, $*p < 0.05$, $**p < 0.001$.

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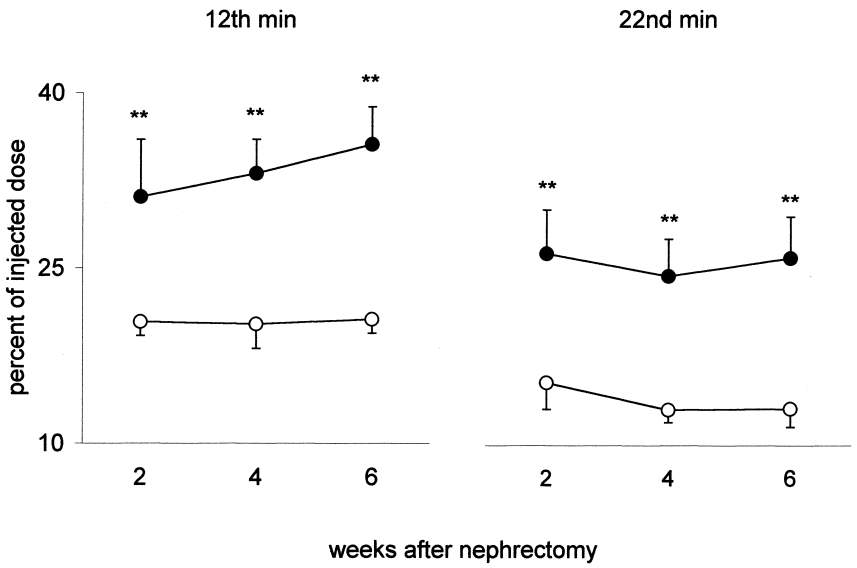

Figure 3. Kidney uptake of ^{131}I-β2-microglobulin (radioactivity of total left kidney, percent of the injected dose) in uninephrectomized rats (●) and in controls (○) 2, 4 and 6 weeks after nephrectomy. On the left the values at the 12th min after i.v. injection of ^{131}I-β2-microglobulin (peak-time); on the right those at the 22nd min. Mean values ± SD, $^{**}p < 0.001$.

or 10 min later (Figure 3). Kidney uptake (per g) of ^{131}I-β2M is also higher in NX than in C at any time after nephrectomy. The difference between NX and C is less relevant than for total kidney but is still statistically significant (Figure 4). This behavior indicates that in the remaining kidney of NX the increase of kidney uptake of ^{131}I-β2M is higher than the increase of kidney weight due to compensatory hypertrophy. The percent difference between NX and C is higher 10 min after the peak, for both, total and 1 g kidney, suggesting a slower release of β2M by the remaining kidney of NX. Kidney concentration capacity of ^{131}I-β2M is very high: the radioactivity of 1 g of kidney is approximately 17 times higher than that of 1 g of plasma (without significant differences between NX and C).

Liver (total and 1g) – Liver uptake of ^{131}I-β2M is much lower than that of the kidney ranging from about 4 to 5.5 percent of the injected dose (total) and from 0.25 to 0.45 (1 g) without difference between NX and C.

Urine (total) – Urine excretion of radioactivity is minimal (approximately 1.20 percent of the injected dose) in both, NX and C.

DISCUSSION

Many low mw proteins of different pI (α1-microglobulin, retinol binding protein, α2U-globulin, α2a-interferon, aprotinin, cytochrome C, lysozyme

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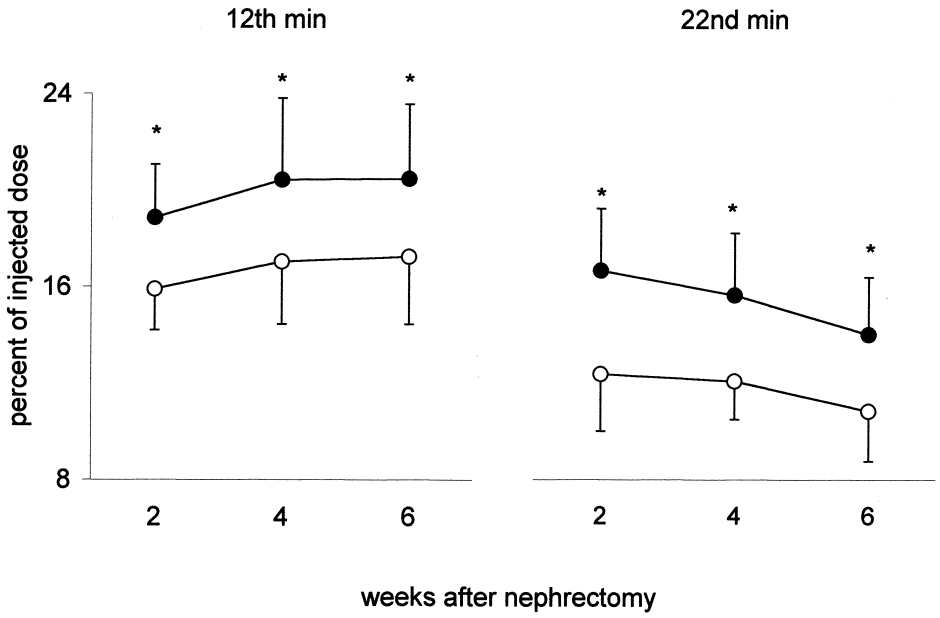

Figure 4. Kidney uptake of ^{131}I-β2-microglobulin (radioactivity per gram of left kidney, percent of the injected dose) in uninephrectomized rats (●) and in controls (○) 2, 4 and 6 weeks after nephrectomy. On the left the values at the 12th min after i.v. injection of ^{131}I-β2-microglobulin (peak-time); on the right those at the 22nd min. Mean values ± SD, $*p < 0.05$.

and β2M) are taken-up and accumulated by the kidney of normal rats (7,15–17).

In man, many low mw proteins seem to be handled by the kidney, as indicated by the increase of their serum levels in renal failure. In particular, this has been demonstrated for β2M, retinol binding protein, lysozyme, complement factor D, α1-microglobulin, cystatin C, tumor associated trypsin inhibitor, prolactin, myoglobin and chromogranin A (9,11,13,18–28). The key role of the human kidney in the handling of low mw proteins is strongly suggested by the large kidney uptake of aprotinin (a 6,500 dalton polypeptide) (29,30). Few data are available concerning the effect of the reduction of renal mass on the renal handling of low mw proteins. Our previous results demonstrate that in the rat uninephrectomy is followed by an increased uptake of radioiodinated α1-microglobulin and lysozyme by the remaining kidney (31,32).

The results of the present study show that the remaining kidney of NX accumulates more ^{131}I-β2M than C and retains it for a longer time. In contrast, liver uptake of ^{131}I-β2M is not influenced by uninephrectomy. Probably the increased kidney accumulation (total and per g) of labeled β2M in NX is due to an increased load per nephron. This can be attributed to the higher plasma ^{131}I-β2M (as demonstrated by our results) and to hyperfiltration, both caused by uninephrectomy.

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The question arises if the increased kidney accumulation of radioiodinated β2M corresponds to an increase in kidney content of endogenous β2M. The results of a study in progress by this group in NX and in C indicate no difference in renal kinetics of ^{131}I-lysozyme and endogenous lysozyme (unpublished data). Therefore it can be assumed that the renal content of endogenous β2M is also augmented, but whether the increased kidney content of β2M (and that of many other low mw proteins) can damage the kidney is not known. It has been postulated that in different experimental situations and in some human diseases, Bence-Jones proteins, lysozyme, interferons, myoglobin and β2M can induce adverse effects on the kidney (33–43). Immunohystologically it has been demonstrated in various renal diseases a link between β2M deposition in renal tissue (tubular epithelium and tubular casts) and tubular interstitial injuries with deterioration of renal function (44). There is some evidence to suggest that filtered bioactive proteins such as insulin-like growth factor 1 may play a role in promoting progressive tubulo-interstitial disease (45).

The above reported data suggest that the reduction of renal mass (uninephrectomy) could produce tubular hypermetabolism of the many low mw proteins filtered and accumulated in excess by the proximal tubular cells. Hypermetabolism could increase renal ammoniogenesis, which can damage the kidney directly or triggering the release of multiple mediators of tissue injury and promoting fibrosis (46–49). Amino-acids derived from an increase in the breakdown of reabsorbed peptides may constitute an important nitrogen pool for ammoniogenesis in this situation (50).

In conclusion, our findings demonstrate that in the rat the reduction of renal mass increases kidney content of β2M. The consequent renal hypermetabolism of β2M (and probably of other low mw proteins) could play a role in the progression of kidney damage.

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