Excretion of Human Urinary Kallikrein Quantity Measured by a Direct Radioimmunoassay of Human Urinary Kallikrein in Patients with Essential Hypertension and Secondary Hypertensive Diseases

Kazuaki Shimamoto, M.D., Nobuyuki Ura, M.D., Shigemichi Tanaka, M.D., Akio Ogasawara, M.D., Takashi Nakao, M.D., Yasuyuki Nakahashi, M.D., Julie Chao, Ph.D.* Harry S. Margolius, M.D.*, and Osamu Iimura, M.D.

Recently, we established a very sensitive, specific and simple direct radioimmunoassay method for human urinary kallikrein. In this study, in order to clarify whether or not the low or high excretion rate of urinary kallikrein activity in patients with essential hypertension, primary aldosteronism, pheochromocytoma and Bartter’s syndrome is caused by changes in enzyme quantity, urinary kallikrein excretion was measured with this direct radioimmunoassay method in normal subjects and in patients with these diseases.

Urinary kallikrein excretion measured as enzyme quantity was significantly lower in patients with essential hypertension, and higher in patients with primary aldosteronism and Bartter’s syndrome.

These results are consistent with other previously reported data and our data measured by means of esterase assay or kininogenase assay. The results also suggest that lowered or elevated excretion of urinary kallikrein activity in these diseases is caused, in part at least, by the lowered or elevated excretion of enzyme quantity.

Although the functions of the urinary kallikrein-kinin system are incompletely defined, there is some evidence that they may be correlated with the regulation of renal blood flow, modulation of tubular salt and water transport, and pathogenesis of a number of human diseases, such as hypertension.

Human urinary kallikrein excretion, determined as esterolytic activity or kininogenase activity, is lower in patients with essential hypertension and higher in patients with primary aldosteronism as compared with that in normal subjects.

Recently, we purified human urinary kallikrein successfully, and established a very sensitive, specific and simple direct radioimmunoassay method for human urinary kallikrein using this purified enzyme. This assay system can also measure kallikrein quantity in human urine.

In order to clarify whether or not the low or high excretion rate of urinary kallikrein activity in patients with essential hypertension, primary aldosteronism, pheochromocytoma and Bartter’s syndrome kallikrein excretion was measured by...
Fig. 1. Antibody-titration curve. The tubes, containing 200 μl of assay buffer, 100 μl of variously diluted antibody, and 100 μl of 125I-labeled human urinary kallikrein (10000 cpm) were incubated at 24°C for 24 hours. After incubation, antibody-bound kallikrein was separated from free enzyme by the PEG method.

Fig. 2. Detailed procedure of the human urinary kallikrein direct radioimmunoassay.

Fig. 3. Log-logit transformation of standard curves (n = 10) of human urinary kallikrein (mean ± 2SD), human urine sample (n = 4)(mean ± SD), rat urinary kallikrein (RUK), hog pancreas kallikrein (HPK), and monkey, dog, rat and mouse urine samples. Ordinates: B/B₀ percent of logit scale; abscissae: log scale of standard quantities in weight or volume.
means of direct radioimmunoassay in normal subjects and in patients with these diseases.

MATERIALS AND METHODS

Measurement of Human Urinary Kallikrein Excretion by Direct Radioimmunoassay

Urinary kallikrein excretion was measured by the previously reported direct radioimmunoassay method. In this assay system, human urinary kallikrein was purified from 200 liters of pooled human urine by hollow fiber ultrafiltration, ammonium sulfate fraction, DEAE cellulose, aprotininagarose affinity and Sephacryl S-200 column chromatography. This purified enzyme was used in the preparation of the antibody and tracer, and standard.

Antibody against human urinary kallikrein was prepared in rabbits. Purified human urinary kallikrein emulsified with an equal volume of complete Freund’s adjuvant was injected into male New Zealand rabbits. Antibody titers reached a plateau 4 months after injection. The antibody titration curve can be seen in Fig. 1. Fifty percent of the added $^{125}$I-labeled human urinary kallikrein was bound using a final dilution of $1:1,400,000$. The antibody was used in a final dilution of $1:2,500,000$. Purified human urinary kallikrein was labeled with $^{125}$I using lactoperoxidase according to the method of Miyachi et al. The specific activity of $^{125}$I-labeled human urinary kallikrein determined by measuring the fractional reduction in binding of labeled enzyme to antibody after the addition of unlabeled human urinary kallikrein was $73.2 \mu$Ci/µg.

Figure 2 shows the detailed procedure of human urinary kallikrein direct radioimmunoassay. The radioimmunoassay procedure was carried out in 12 x 75 mm polystyrene tubes. Phosphate-buffered saline (PBS; 0.14 M NaCl in 0.01 M Na$_2$HPO$_4$-NaH$_2$PO$_4$, PH 7.0) containing 1% bovine serum albumin was used as the assay buffer. The volume of the assay buffer including the purified standard human urinary kallikrein (8–1000 pg) or samples (0.5–2 µl) totaled 200 µl. The antibody was diluted 1:625,000 with the assay buffer, and 100 µl was added to each tube. The radio-iodinated enzyme (approximately 10,000 cpm) in 100 µl assay buffer was added, making the final incubation volume 400 µl and the final antibody dilution 1:2,500,000. All samples or standard human urinary kallikrein were assayed in duplicate. Tubes were incubated at room temperature (approximately 20°C) for 24 hours. Antibody bound kallikrein was separated from free kallikrein by means of a polyethylene glycol (PEG) method. On percent of bovine gamma globulin in PBS (400 µl; 4°C) and 25 percent PEG (800 µl; 4°C) were added to the tubes and mixed. The tubes were centrifuged at 4000 rpm for 20 min at 4°C. The supernatant was then aspirated and the radioactivity in the unwashed precipitates was counted in an Aloka Auto-gamma Spectrometer. The control tubes contained no standard kallikrein or sample. Blank tubes contained no antibody. Precipitate counts (B) were expressed as a percent of the control tube counts (B₀) after blank tube counts were subtracted from both. Fig. 3 shows the log-log transformation of standard curves of purified human urinary kallikrein, rat urinary kallikrein, hog pancreas kallikrein, and human, rat, mouse, dog or monkey urine samples. In this assay system, the displacement curves for human and monkey urines were parallel to the human urinary kallikrein standard curve. The sensitivity of this assay was 8 pg/tube.

Human Urine Samples

Urine samples were obtained from 17 normal subjects (6 females and 11 males, aged 20–65 years; mean ± SE, 40.5 ± 3.6 years), 52 patients with essential hypertension (23 females and 29 males, aged 20–70 years; 47.0 ± 1.6 years), 4 patients with primary aldosteronism (4 females, aged 32–52 years; 41.5 ± 5.0 years), one patient with pheochromocytoma (male, aged 18 years) and one patient with Bartter’s syndrome (male, aged 40 years). In these patients, all hypertensive and special drugs were withdrawn for at least 10 days prior to the measurement. Twenty-four hour urine samples were collected daily for 3 days to measure kallikrein quantity in both normal subject and patient groups. Urinary kallikrein excretion was expressed as the average on the 3 days.

Statistical Analysis

For unpaired data, statistical analysis was performed employing Student’s t-test with a Canon BX-1.

RESULTS

The results of the determinations of human urinary kallikrein quantity in normal subjects and in subjects with various diseases are shown in Fig. 4.
Fig. 4. The excretion of urinary kallikrein quantity (μg/day) in 14 normal subjects, 52 patients with essential hypertension, 4 patients with primary aldosteronism, 1 patient with pheochromocytoma and 1 patient with Bartter's syndrome.

Urinary kallikrein excretion was 136.5 ± 22.5 μg/day, mean ± S.E., in 17 normal subjects. This enzyme excretion was significantly lower in 52 patients with essential hypertension, 70.8 ± 6.2 μg/day, and higher in 4 patients with primary aldosteronism, 366.1 ± 56.7 μg/day, than that in normal subjects.

In the patient with pheochromocytoma, the urinary kallikrein excretion was 256.1 μg/day. In the patient with Bartter's syndrome, this excretion showed a remarkably higher levels, 948.9 μg/day, than in normal subjects.

DISCUSSION

Urinary kallikrein has been measured primarily as an enzyme activity by means of esterase assay5-9 or kininogenase assay. These methods have provided some insight into urinary kallikrein levels in normal10,16,17 and diseased subjects.

However, all these methods which estimate kallikrein activity in urine are subject to interference from kallikrein inhibitors. Indeed, kallikrein inhibitors were recently observed in human urine. For this reason, radioimmunoassays, able to measure kallikrein concentration rather than activity, are desirable for measurement of enzyme protein quantity. Direct radioimmunoassays have been reported in the rat by Carretero et al (1978) and by us20 (1979). In addition, more recently, we established a very sensitive, specific, and simple radioimmunoassay method for human urinary kallikrein. Therefore, in this study, the excretion of human urinary kallikrein quantity was measured with this direct radioimmunoassay in normal control subjects and in essential hypertension, primary aldosteronism, pheochromocytoma and Bartter's syndrome patients (Fig. 4).

Human urinary kallikrein excretion was significantly lower in patients with essential hypertension than that in normal subjects. This data is consistent with other previously reported data (including our data) which were measured as esterase or kininogenase activity.5-7,12 These
results suggest that lowered excretion of urinary kallikrein activity is caused at least partially by the lowered excretion of enzyme quantity. On the other hand, urinary kallikrein quantity excretion was significantly higher in primary aldosteronism and Bartter's syndrome patients than in normal subjects. These results are also consistent with other data measured by means of esterase assay.\textsuperscript{5,6,21}

We already reported that the urinary excretion of kallikrein quantity measured with direct radioimmunoassay showed very similar results to those obtained by esterase assay in normal subjects given altered sodium intake or in normal black and white children, and that the specific activities of kallikrein (milliesterase unit/mg enzyme) were not significantly different under those conditions. Our data show that changes in the urinary excretion of kallikrein quantity are also similar to those estimated by esterase assay or kininogenase assay in these disease states. However, we have not yet finished measurement of kininogenase activity and esterase activity in the same samples. Simultaneous determination by kininogenase assay and the direct radioimmunoassay is very useful in obtaining a detailed picture of the urinary kallikrein-kinin disorder in essential hypertension or other diseases. Further study is necessary, however, to clarify the role of the renal kallikrein-kinin system in these diseases.

Since our direct radioimmunoassay for human urinary kallikrein did not show a cross reaction against human urinary prokallikrein, we also established a very simple method for measurement of human urinary prokallikrein.\textsuperscript{22} Thus far, both measurements of urinary kallikrein by esterase assay, kininogenase assay and direct radioimmunoassay, and that of prokallikrein by our method in combination with measurements of urinary kinin,\textsuperscript{11} urokininogen\textsuperscript{23} and kininase activity\textsuperscript{24} by kinin and/or angiotensin-II radioimmunoassay\textsuperscript{11,25} have proven a very useful investigation of the role of the renal kallikrein-kinin system in normal or diseased states.

This kind of comprehensive work will be very helpful in clarification of the renal kallikrein-kinin system's role.

Acknowledgement

The authors are indebted to Miss M. Korenaga for her technical assistance and Miss Y. Kawakita for her secretarial assistance.

REFERENCES


NII-Electronic Library Service
Endocrinol Metab 33: 732, 1971