Clinical Application of Basic Arginine Amidase in Human Male Urine

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Basic human urinary arginine amidase (or esterase, called BHUAE) which is only found in male urine, was measured from normal volunteers between the age of 4 and 70 years using D-valyl-L-leucyl-L-arginine-*p*-nitroanilide as a substrate. BHUAE increases during early adolescence, between 8 to 17 years of age. Then, BHUAE decreases in the twenties and takes a certain range of value in the mature age group, between the late thirties and fifties.

In patients with prostate cancer, a significant increase in BHUAE was demonstrated in comparison with the healthy male group (control) over 55 years old. On the other hand, patients with benign prostatic hypertrophy showed no significant elevation of this enzyme activity. It would appear that the measurement of BHUAE in urine can be used as a marker of prostate cancer in an advanced age group.

Key words human male urine; basic human urinary arginine amidase (BHUAE); prostate cancer; benign prostatic hypertrophy; prostate specific antigen (PSA)

Mammalian urine contains some arginine amidases that have certain physiological and pharmacological functions, such as tissue kallikrein (EC 3.4.21.35),^{1,2)} urokinase (EC 3.4.21.69)^{3,4)} and acidic arginine esterase(amidase).^{5–7)} These enzymes are found in the urine of both sexes of mammals.

On the other hand a basic arginine esterase (amidase) with a basic protein molecule (so-called Esterase A-1), reported by McPartland *et al.*,⁸⁾ is detected only in male urine. Thus enzyme in rat and human urines was later purified and some of its properties were characterized.^{9,10)} This enzyme may originate in the male sub-sexual organs, such as the prostate, for it was not found in male urine collected from the ureter and bladder.⁹⁾ We separated some basic arginine amidases from the prostate, testis, seminal plasma and urine of dogs, by lima bean trypsin inhibitor (LBTI) and aprotinin affinity adsorptions, examined the activity content per tissue, and found particularly high levels of activity in seminal plasma and prostate.¹¹⁾ Furthermore, we also purified basic arginine amidase in human seminal plasma, which is considered to be an origin of basic arginine amidase in human male urine, by LBTI affinity column, and characterized its properties.¹²⁾

In the present paper we examined the clinical applications of basic arginine amidase (BHUAE) in human male urine.

MATERIALS AND METHODS

Human Male Urine The urine samples were collected from healthy normal volunteers and inpatients who visited Sanraku Hospital and Teikyo University Hospital with prostate cancer and benign prostatic hypertrophy, and were stored frozen until use. The age of all patients with prostate cancer and benign prostatic hypertrophy was 59 years old and older.

Assay of Arginine Amidase Activity The measurement

of D-valyl-L-leucyl-L-arginine-*p*-nitroanilide (Val-Leu-Arg-*p*NA) hydrolytic activity by basic and acidic arginine amidases (esterases) in human male urine was done using the slightly modified method of Amundsen *et al.*,¹³⁾ with 0.5 mM of substrate concentration in 0.05 M Tris-HCl at pH 8.5 for 37 °C. The arginine amidolytic activity was expressed in terms of nmol of substrate hydrolyzed per minute per milliliter of original male urine (nmol/(min·ml) urine) or nmol/(min·d) of urine, under the above mentioned conditions, after measuring the absorbance at 410 nm of *p*-nitroaniline (*p*NA), which was subsequently released by the amidolytic enzyme reaction from the substrate using a Hitachi Clinical Spectrophotometer.

Separation Procedure of Basic Arginine Amidase from Human Male Urine The separation procedure of basic arginine amidase (esterase, BHUAE) preparation from human male urine was carried out at room temperature in a way similar to that described previously by us¹⁰⁾ as follows.

Human male urine (100 ml) was dialyzed against de-ionized water and 0.02 M Tris-HCl buffer at pH 7.5, and then centrifuged with cooling. The conductivity and pH of the dialyzed human male urine were adjusted to 1 mS/cm or less, and pH 7.5, respectively, and then applied to a small CM-cellulose column (1 × 10 cm), pre-equilibrated with the above-mentioned buffer, and the column was washed with the same buffer. The elution of basic human urinary arginine amidase preparation was made with 0.05 M Tris-HCl buffer at pH 8.5 containing 0.5 M NaCl, and the active fractions (about 5 to 10 ml) were collected. Using this procedure, acidic arginine amidases, which adsorbed to DEAE-cellulose, such as tissue kallikrein and HUAE showing Val-Leu-Arg-*p*NA amidolytic activity and trypsin inhibitor, are expected to be removed. The collected specimens were measured for enzyme activity as described above.

The basic arginine amidases in human male urine could be separated into two enzyme preparations with different

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molecular masses, 4.5 and 1.8×10^4 daltons, by Cellulofine GCL-2000 gel filtration procedure, after treatment using the above-mentioned procedure.¹⁰⁾

Statistical Analysis Data is shown as the mean $\pm$ S.D., and the coefficient correlation test was carried out using the method of a parametric test with StatView J 4.5 for the Macintosh computer.

Materials The materials, including chromatographic materials, substrate, and chemicals used in this investigation, were obtained commercially and were of analytical reagents grade.

RESULTS AND DISCUSSION

The results of measurement of BHUAE activity in males between 4 and 68 years of age, without prostate diseases, are summarized in Fig. 1. Samples taken from the age 55 and older persons are indicated as the 55 year and older group. BHUAE activity remains at a relatively low level up to around 7 years of age (infantile age), then rises between early adolescence at 8 years old, and the sexually matured age of around 17 years, in when the male hormone reaches its highest value, with the peak value being $18.2 \text{ nmol}/(\text{min} \cdot \text{d})$ (Fig. 1). Level change patterns

Fig. 1. Age Excretion of BHUAE Activity in Human Male Urine

BHUAE activity is expressed in terms of $\text{nmol}/(\text{min} \cdot \text{d})$ of Val-Leu-Arg-*p*NA hydrolysis.

between the BHUAE activities found in this investigation and the testosterone contents reported by Lee *et al.*¹⁴⁾ in the blood of adolescent boys are quite similar. The enzyme activity starts to decline between 17 and 18 years of age and stabilizes at a certain range in the mature age between 35 and 50 years old, with a mean value of $1.3 \pm 1.3 \text{ nmol}/(\text{min} \cdot \text{d})$ ($n=15$). The mean value for the healthy 55 and older group, except for the highest one, is $1.4 \pm 1.1 \text{ nmol}/(\text{min} \cdot \text{d})$ ($n=12$), which was not significantly different from that of the mature age group between 35 and 50 years old.

BHUAE in the urine of men is influenced by testosterone, and the enzyme is originated in male sub-sexual organs—the prostate in particular, because the prostate is an organ containing large amounts of basic arginine amidase activity¹¹⁾ and the enzyme activity appears to be dependent upon prostate diseases, as discussed later in Fig. 2. Marshall and Tanner¹⁵⁾ reported that the male reproductive function reaches near full maturity at around 17 years of age—the near-completion of adolescence. Thus, the highest activity of BHUAE in human male urine at an age of 17 to 18 years, and the subsequent decline, can be explained as follows: once male reproductive function becomes mature in later adolescence, testosterone requirements by sexual organs, including the prostate, decrease, thus lowering the level of testosterone in the blood after the twenties. This seems to cause the enzyme activity to decline. A similar conclusion can be drawn from the finding that the enzyme activity remains at a low level in the mature age (around 35 or older) during which male reproductive function deteriorates.

BHUAE activity was measured in the urine of patients with prostate cancer and in benign prostatic hypertrophy patients within the 55 years or older age group. The Val-Leu-Arg-*p*NA amidolytic activity of benign prostatic hypertrophy patients is $3.1 \pm 2.2 \text{ nmol}/(\text{min} \cdot \text{d})$ ($n=6$), not significantly different from that observed in the healthy group ($1.4 \pm 1.1 \text{ nmol}/(\text{min} \cdot \text{d})$, $n=12$). On the other hand, the activity of the prostate cancer patient group is $15.4 \pm 8.6 \text{ nmol}/(\text{min} \cdot \text{d})$ ($n=15$), which is 11 times as high as that of the healthy group of 55 years or older, which is a significance difference ($p < 0.02$) (Fig. 2). These results support our previous discussion that BHUAE may originate in the prostate gland.⁹⁾ In the patient with prostate cancer, the parenchymatous part of the prostate

Fig. 2. Excretion of BHUAE Activity in Human Male Urine of Patients with Some Prostate Diseases

BHUAE activity is expressed in terms of $\text{nmol}/(\text{min} \cdot \text{d})$ of Val-Leu-Arg-*p*NA hydrolysis.

Fig. 3. Relationship between BHUAE Activity and PSA Amount in Patients with Prostate Cancer

BHUAE activity and PSA amount are expressed in terms of pmol/(min·ml) urine of Val-Leu-Arg-pNA hydrolysis and ng/ml of blood, respectively.

is cancerized and hypertrophied, showing the presence of the enzyme in the parenchymatous part. BHUAE may thus be useful as a marker of prostate cancer in the advanced age group.

Patients with transurethral resection of the prostate, at two and more weeks after the operation, also show high BHUAE activity of 40.7 ± 13.2 nmol/(min·d) ($n=6$) (Fig. 2). This value is 19 times higher than that of the healthy group of 55 years or older, which is a significant difference ($p<0.02$). It would appear that BHUAE is directly secreted from the parenchymatous part of the prostate through the urethra. However, the high activity of BHUAE in patients with a transurethral resection of the prostate is not able to be explained at this stage. Future research is being conducted on these subjects.

The relationship between BHUAE present in human urine and acidic arginine amidase in human urine (HUAE), which has no kinin releasing activity, making it different from tissue kallikrein,^{1,2)} is measured in 16 samples, including 7 prostate cancer samples. The HUAE is found in the urine of either sex,⁵⁾ and there is no significant difference, because HUAE may originate in the kidney⁵⁾ and its activity is significantly increased by the primary aldosteronism of kidney disease.⁶⁾

The prostate gland, plasma and seminal plasma contain a prostate-specific antigen called PSA,¹⁶⁾ and the PSA has chymotrypsin-like amidolytic activity.^{17,18)} However, the PSA has no arginine amidase activity.^{18,19)} BHUAE

and PSA seemed to be different proteins according to their enzymatic properties. However, there are still some similarities between both materials, such as similar molecular masses.^{10,12,16)} Even so PSA increases in benign prostatic hypertrophy,²⁰⁾ while BHUAE activity is not increased by this disease (Fig. 3). We examined patients with prostate cancer for PSA in plasma and BHUAE activity, and the correlation coefficient was $r=0.733$ (9 specimens, $p<0.05$) (Fig. 3). Further studies on the relationship between the BHUAE and the PSA should be conducted in the near future.

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