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Review

Chromatography of guanidino compounds

Toshihiko Hanai[a,*], Yukiko Inamaoto[b], Shigeyuki Inamoto[b]

[a]*Health Research Foundation, Institut Pasteur 5F, Sakyo-ku, Kyoto, 606-8225 Japan*
[b]*Teikoku Seiyaku Co., Sanbonmatsu, Ochi-cho, Okawa-gun, Kagawa, 769-26 Japan*

Abstract

Guanidino compounds involved in the urea and guanidine cycles have been found in serum of nephritic patients, and some guanidino compounds have been suspected to be uremic toxins. The simultaneous analysis of naturally occurring metabolites is important for diagnosis of diseases. In this review, liquid chromatographic analysis of natural metabolites of guanidino compounds are described. the information about arginine as a precursor of nitric oxide are included. The reports of pharmaceutical compounds having a guanidino group, peptides containing arginine and aminoglycosides are summarized in Table 1.

Contents

1. Introduction

Guanidino compounds involved in the urea and guanidine cycles have been found in serum, and several are uremic toxins. The simultaneous analysis of naturally occurring metabolites is important for diagnosis of diseases. Arginine is a precursor of nitric oxide and a variety of N^G-methyl arginines derivatives are potent inhibitors of nitric oxide synthase. The guanidino group is very basic, and these compounds are usually ionized in vivo. Therefore, these guanidino compounds are separated by liquid chromatography for analytical and purification purposes. In this review, the details of natural metabolites of guanidino compounds are described. The information about arginine as a precursor of

*Corresponding author. Tel.: +81-45-402-5861; fax: +81-45-402-6361.
E-mail address: thanai@ibm.net (T. Hanai).

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nitric oxide are included. In addition, the reports of pharmaceutical compounds having a guanidino group, peptides containing arginine and aminoglycosides are summarized in Table 1.

2. Guanidino compounds in metabolic pathway

Metabolism may be defined as the sum of the processes by which living matter is built up or broken down. It considers both the chemical reactions needed to provide materials and energy for growth, maturation, reproduction and homeostasis, and the ways in which such reactions are controlled. The research interest has considered the mechanisms by which these reactions are controlled and modulated. Therefore, the simultaneous analysis of metabolites is the best method to study the reaction activities. The analysis of naturally occurring metabolites can lead to the prevention of metabolic disorders and to the identification of compounds prognosis of cancer, cardiovascular diseases, diabetes, etc. The metabolic pathways of guanidino compounds are shown in Fig. 1.

Several guanidino compounds have been found in serum of nephritic patients [1], and some guanidino compounds have been suspected to be uremic toxins. Methylguanidine was shown to be related to the uremic polyneuropathy found in uremia [2]. Guanidinosuccinate was related to uremic bleeding diathesis, and was shown to inhibit excitatory neurotransmission in rat hippocampal brain slices, an effect that hypothetically could contribute to uremic encephalopathy [3]. Guanidinosuccinate, methylguanidine, guanidine and creatinine were suggested to be the cause of chronic and generalized seizures after systemic and intracerebroventricular administration in mice [4,5]. γ-Guanidinobutyric acid, methylguanidine, taurocyamine, creatine and creatinine were found to have a convulsive effect in animals when administered intracisternally. Furthermore, in vitro studies have shown that guanidinosuccinic acid, γ-guanidinobutyric acid, guanidinoacetic acid and β-guanidinopropionic acid might be factors responsible for the increased hemolysis. Methylguanidine and creatinine were shown to induce hemolysis both in vitro and in vivo. Guanidinosuccinic acid inhibits erythrocyte transketolase and methylguanidine inhibits brain sodium-potassium ATPase. A variety of guanidino compounds increase in blood, urine and cerebrospinal fluid of nondialyzed as well as hemodialyzed uremic patients [6–8]. The reference intervals (mean±2SD) erythrocyte creatinine obtained from 60 males and 60 females were (in μmol/g hemoglobin) 1.18±0.52 (0.66–0.70) for males and 1.35±0.49 (0.86–1.84) for females [9]. Guanidino compounds in serum of chronic renal failure patients was monitored to study the effects of oral adsorbent AST-120 [10]. Creatinine and related compounds have been analyzed enzymatically and chromatographically [11–22]. Arginine was analyzed using on-line indirect chemiluminescence detection in capillary electrophoresis with other amino acids [23]. Hyponatremia was induced in rats with 1-desamino-8-D-arginine vasopressin. The brain organic osmolytes (glutamine, myo-inositol and taurine) were monitored to study the effect of prior hyponatremia on the development of ammonia-induced brain edema in rats after portacaval anastomosis [24].

The effects of clinical dialysis have been studied by measuring the guanidino compounds by liquid chromatography [25]. The serum guanidino compound levels found in uremic children were comparable to those in adults. A single hemodialysis lowered the level of most serum guanidino compounds temporarily, and the level remained more stable in continuous cycle peritoneal dialysis-treated patients [7]. A mechanistic explanation for the differences in the extent of decreases in the levels of different guanidino compounds was not yet available. No correlation was reported between the molecular mass or the acidity of the guanidino compounds and their percentage decrease. The guanidino compounds were distributed throughout the tissues and their protein binding, in combination with other factors, contributes to the differences in their dialysability [8]. Several guanidino compounds are included in the extensive list of possible uremic toxins [26]. Therefore, the development of rapid and sensitive analytical method was required. Methylguanidine and guanidinoacetate are measured by enzyme activity assays; however, the sensitivity of the assays is not satisfactory for the measurement of these compounds in blood [27]. The enzymatic analysis methods for the analysis of all available

Table 1
Pharmaceutically important compounds

	Reference
Drugs	
Antifungal macrolide antibiotic AK-165-3	[110]
Apromidine type histamine	[102]
Arginyltransferase	[106]
Benzyl guanidine	[101]
Buformin	[96]
Cimetidine	[104]
2,2′-Diamino-4,4′-dithiazole derivatives	[103]
Guanabenz	[97,98]
Guanethidine sulfate	[95]
Guanidine analogues	[108]
Guanidinocontaining drugs	[111]
Guanfacine	[99]
Guanoxabenz	[98]
Heptanamide	[105]
meta-Iodobenzyl guanidine	[101]
Mefruside	[97]
Metformine	[96]
Minalemines A–F	[109]
Monamidocin	[107]
N-(3-Nitroquinoline-4-yl)morpholinocarboxamidine	[100]
Phenformin	[96]
Aminoglycoside antibiotics	
Amikacin	[112]
Aminoglycoside residues	[113]
Azithromycin	[125]
Dihydrostreptomycin	[114,117,118,121]
Gentomycin	[112]
Knamycin	[112]
Neomycin	[112,115]
Netilmicin	[112]
Novobiocin	[115]
Sisomicin	[112]
Spectinomycin	[112]
Streptomycin	[112,115,116] [119,120,122–124]
Tobramycin	[112,126]
Peptides	
N-Arginine dibesic convertase	[132]
Angiotensin	[199,200]
Bradykinin	[200–202]
Buserelin	[172]
Calcitonin	[188–198]
Delargin	[211]
Desmopewaain	[211]
Dynorphin B	[203]
Endorphin	[203]
Enkephalin	[207]
Exogegenous peptides	[204]
Fusaricidin	[146]
Gonadorelin	[172–176]

Table 1. Continued

	Reference
Gonadotropin	[178–187]
Goserelin	[172,177]
Human growth hormone	[147–171]
Interleukin	[212]
Neocarzinostatin	[209,210]
Neurohypophyseal peptides	[133]
Neurokinin A	[206]
Oxytocin	[137,142]
Peptides	[127–131,134,135]
Pneumadin	[213]
Protein variants	[205]
Thrombin	[145]
Triptorelin	[172]
Vasopressin	[136–141,143,144,208]

guanidino compounds lack sensitivity. Chromatographic methods are useful for the analysis of such mixtures with high sensitivity. However, such methods are time concerning and reproducibility was not satisfactory. The problem of reproducibility was overcome by using a graphitic carbon column which is chemically and physically stable [28,29]. Furthermore, a rapid analytical method was developed using a single eluent with a column switching system. The system was applied to analyze guanidino compounds in sera from nephritic patients. The concentration of guanidino compounds was correlated among metabolites after divided by the values of creatinine to study the specification of diseases and metabolic pathway.

2.1. Chromatography of urea and guanidino cycles guanidino compounds

Guanidino compounds are very polar, usually exist as ionic form. The separation is usually carried out by ion-exchange liquid chromatography [25,30–32] or reversed-phase ion-pair liquid chromatography [33,34]. However, the separation has not been satisfactory. Prederivatization was applied using benzoin

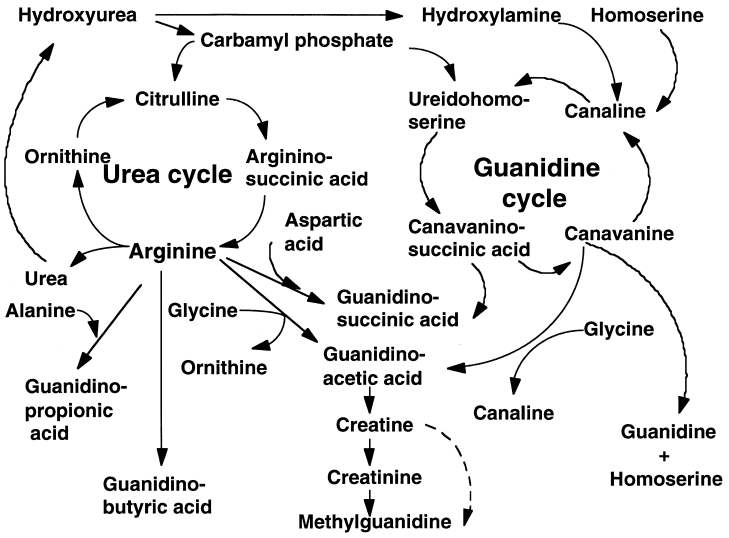

Fig. 1. Metabolic pathway of guanidino compounds.

[35–37] and ninhydrin [38]. Water-soluble 9,10-phenanthrenequinone-3-sulfonate was used for the fluorometric determination [31]. Guanidino compounds were determined by anion-exchange liquid chromatography and amperometric detection. The detection limits were 3 to 12 pmol [39]. Creatinine was analyzed by gas chromatography–mass spectrometry [40]. Creatinine, creatine and guanidinoacetic acid in human serum and urine were simultaneously determined by liquid chromatography–atmospheric-pressure-chemical-ionization mass spectrometry (LC–APCI-MS) [41]. However their separation and reproducibility have not been satisfactory due to instability of packing materials and the complicated separation systems, furthermore, the highly sensitive post-column reaction detection is preferred to pre-column derivatization for their quantitative analysis.

Porous graphitic carbon of small particle-size was developed by Knox et al. [28,29], and later Obayashi et al. [42] and Ichikawa et al. [43]. Porous graphitic carbon is stable in strong acids and alkalis solutions, and therefore, eluents at any pH can be used. Porous graphitic carbon is extremely hydrophobic, and the retention time of ordinary compounds was quite long compared to that of the commonly used octadecyl-bonded silica gels. Therefore, porous graphitic carbon is suitable for the chromatography of very polar compounds, e.g., saccharides [44–46] and ions [47–49]. The retention mechanism is considered as a mixture of hydrophobic and electrostatic interactions [50], and electrostatic interaction is seemed to be more important than hydrophobic interaction in the retention mechanism of polar compounds. Therefore, Inamoto et al. studied separation of guanidino compounds using porous graphite carbon columns [51].

2.1.1. Optimization for retention of guanidino compounds

The pH and metal ion (lithium, sodium and potassium) effects in citrate and phosphate buffers were studied using a TSKgel Carbon-500 column in citrate buffer from pH 3.00 to 12.00 and phosphate buffer from pH 3.00 to 12.90. The smaller the size of metal ion, the longer the retention; however, increasing the concentration of buffer up to 200 m*M* did not affect their retentions. Sodium acetate and rubidium acetate buffers were also examined, but their retention times were equivalent to those in the sodium citrate buffer. Strong retention of the compounds was observed in the pH 4.50 citrate buffer, and in the high pH phosphate buffer. The former retention mechanism may be due to electrostatic interaction, and the latter retention mechanism due to hydrophobic interaction. These guanidino compounds were scarcely retained on octadecyl- and octyl-bonded silica gels (ODS & C8) in the 50 m*M* sodium citrate buffer at pH 3.00–10.00. The separation of these compounds was possible with the pH 4.50 citrate buffer on the graphitic carbon column (where these compounds are completely ionized), but the total separation was difficult.

Ion-pairing effects were studied mainly in the pH 4.50 citrate buffer for the total separation of guanidino compounds. Several ion-pair reagents (5 m*M*) were added to the 50 and 100 m*M* sodium citrate buffer at pH 4.50. Octanesulfonate was the most effective ion-pair reagent in the 100 m*M* sodium citrate buffer at pH 4.50. The concentration effect of octanesulfonate was studied in 50 m*M* sodium citrate buffer at pH 4.50. Increasing the concentration of octanesulfonate basically increased the retention factors; however, increasing the concentration made the operation difficult. Therefore, 5 m*M* was selected as the final concentration of octanesulfonate for subsequent studies. The concentration effect of sodium citrate buffer was examined with a pH 4.50 solution containing 5 m*M* octanesulfonate.

The retention factors of guanidino compounds were increased in less than 20 m*M* of sodium citrate buffer, and the retention times of some compounds exceeded over 1 h (except the very polar compounds). The addition of organic modifier decreased the retention time. The addition of 5% (v/v) of methanol cut the retention time in half. The baseline separation of the ten guanidino compounds was successful using a gradient elution from Eluent A (10 m*M* sodium citrate buffer at pH 4.50 containing 5 m*M* octanesulfonate) to Eluent B (10 m*M* sodium citrate buffer at pH 4.50 containing 20% acetonitrile) on a BTR carbon column. Examples of the chromatograms of standard guanidino compounds are shown in Fig. 2. The detection limit was measured by the injection of 50 μL of sample solutions, and the value of guanidino compounds was 7.8 ng/ml

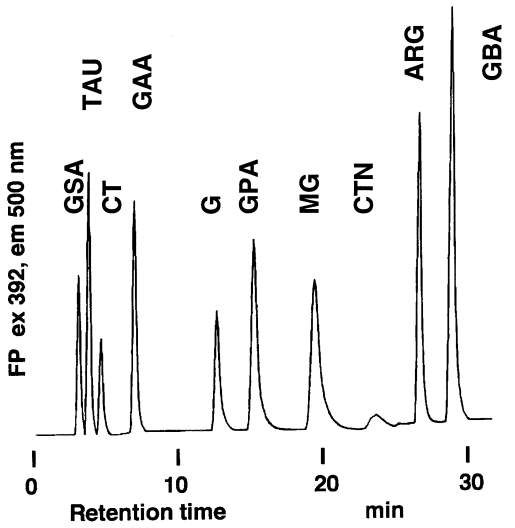

Fig. 2. Separation of ten standard guanidino compounds on a porous graphite carbon column in ion-pair liquid chromatography. Column: 50×4.6 mm I.D. packed 3.5 μm graphitic carbon. Eluent: Four step-wise gradient from 10 m*M* sodium citrate buffer (pH 4.50) containing 5 m*M* sodium octanesulfonate to 10 m*M* sodium citrate buffer (pH 4.50) containing 20%(v/v) acetonitrile (see the details in text); flow rate: 1.0 ml/min at 40°C, flow rate of 0.6% ninhydrin and 1 *M* NaOH solns.: 0.2 ml/min.

(S/N=3), (except for creatinine), and their calibration curves were linear up to 2 μg/ml (r^2=1.000). The detection limit of creatinine was 1 μg/ml, about one-hundredth of those of the other guanidino compounds. The recovery of these guanidino compounds from serum was more than 90% (except for creatinine).

The stable system was applied to the analysis of guanidino compounds in serum from nephritic patients such as diabetic nephropathy (DMN), membraneproliferative glomerulonephritis (MPGN), chronic glomerulonephritis (CGN), continuous ambulatory peritoneal dialysis after renal transplantation (CAPD-RT), gouty kidney (GK), focal glomerular sclerosis (FGS), hypertension (HT), IgA glomerulonephritis (IgAGN), nephrotic syndrome (NS) and membranous nephropathy (MN). Blood samples collected from humans were centrifuged at 1000 *g* for 15 min at room temperature, and the sera were stored at −30°C. The 300 μl samples of sera were filtered with Minicent-10 (Tosoh) by centrifugation at 3000 *g* for 20 min. Aliquots (50 μl) of the filtrates were injected into the analytical system.

Examples of the chromatograms of a healthy control subject, a CAPD-RT patient and a MPGN patient are shown in Fig. 3A–C. GSA, G, GPA and MG were found in sera from the patients but not in sera from the controls. The amount of CT, GAA and GBA were large in the sera from the controls, and that of CTN and ARG were doubled in the sera from the patients. However, the existence and amount of GSA, G, GPA and MG were not related to the

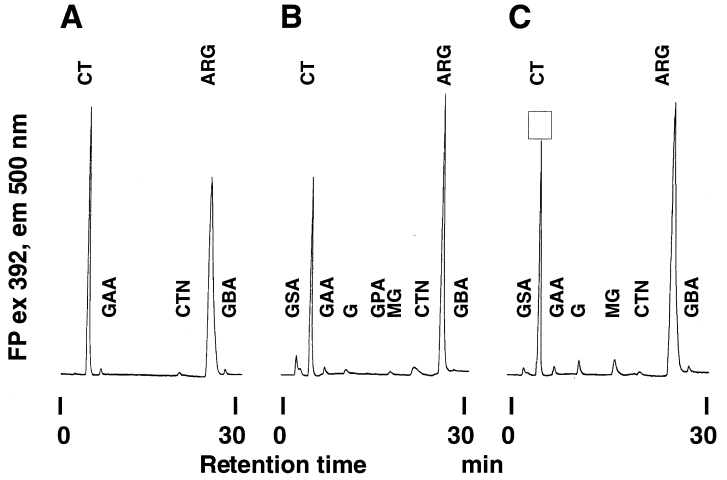

Fig. 3. Analysis of guanidino compounds in sera from A (control) B (CAPD-RT) and C (MPGN). Chromatographic conditions: see Fig. 2.

diseases of these patients. That is, their enzyme activity may not be related to these nephritis [51].

The chromatographic results were not related to the values of blood urea nitrogen (BUN), creatinine (CTN) and uric acid (UA) of patients as measured by routine clinical methods. The presently available enzyme activity methods, with their low sensitivity, can handle only methylguanidine and guanidinoacetate, and the chromatographic analysis of guanidino compounds is therefore important as a quantitative analysis in serum. A liquid chromatograph system equipped with a graphitic carbon column and a post-column labeling detector was a reliable system for analyzing trace levels of guanidino compounds in human sera as a routine analytical method, due to the chemical and physical stability of the graphitic carbon column and the sensitivity of the detector.

2.1.2. Rapid analysis using column-switching with isocratic elution

Biomedical use of liquid chromatography requires rapid separation with a simple procedure and high sensitivity. Therefore, the column switching technique [52] was examined [53]. The purpose of the first column is sample purification, deproteinization, pre-concentration and heart-cut. An aqueous size-exclusion column with a low molecular weight size-exclusion limit can be used for the separation of proteins (large molecular mass compounds) and analytes (small molecular mass compounds). After the elution of proteins, the small molecular weight fraction is transferred to an analytical column, usually a reversed-phase column, and then the mixture of small molecules is further separated [54–56]. Internal surface reversed-phase columns are also used for deproteinization, and analytes are further separated based on their hydrophobicity [57,58]. After the elution of proteins, the fraction containing the target compounds is transferred to an analytical column, usually a reversed-phase column. Occasionally, the analytical column is a chiral separation column. The disadvantage of this method is the adsorption of protein fragments. A small reversed-phase column is used for sample pre-concentration [59–64]. The pre-concentrated analytes are washed with solvent, then back-flushed into the analytical column. This method allows the injection of a large volume of sample solution into a pre-concentration column. However, the complete removal of adsorbed proteins is difficult, and frequent replacement of the pre-concentration column is necessary.

A variety of gradient elution methods are commonly used for the separation of complex mixtures, but gradient elution is not suitable for routine analysis due to the time consuming re-equilibration of the system prior to injection. Multi-column separation methods have not been used since double-column amino-acid analyzer was used. Hirukawa et al. developed a method for column switching separation of a mixture of polar and non-polar substances with isocratic elution for separation of free amino acids where three type of columns were used with one eluent. The separation system was simple even though a variety of pumps, detectors and switching valves were required, and continuous injection was performed without equilibration time [65]. However, such systems have not been widely used because selection of a suitable pair of columns is difficult. Therefore, isocratic elution of guanidino compounds was studied using different types of columns. Furthermore, the liquid chromatographic system was applied to analysis of guanidino compounds in serum from nephritic patients including diabetic nephropathy (DMN), chronic glomerulonephritis (CGN) and sclerotic kidney (SK) [53].

The analytical system for guanidino compounds based on a liquid chromatograph with column switching and a post-column reaction detector is shown in Fig. 4. Direct injection of serum samples was studied using four type columns, i.e. a TSKprecolumn BSA-ODS (bovine serum coated octadecyl column), a Capcell pak MF (surface polar column), a TSKprecolumn PW (polyvinylalcohol gel column for size-exclusion chromatography) and an Asahipak GS-310M (aqueous-phase size exclusion column), using 10 m*M* sodium citrate buffer (pH 4.50) with and without octanesulfonate as the eluent. However, some of these guanidino compounds were polar were co-eluted with serum proteins from these columns, and direct injection into the small size columns was difficult.

These guanidino compounds were not retained on an octadecyl-bonded silica gel column in pH 3–10 50 m*M* sodium-citrate and phosphate buffers. The electrostatic effect at pH 4.50 and hydrophobic effect at pH 10.0 were not observed on octyl and

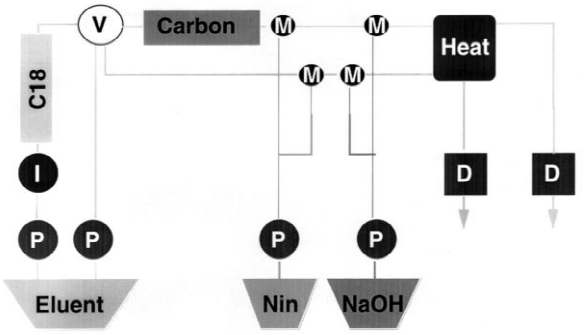

Fig. 4. Chromatographic system for guadinino compounds with single eluent and two columns. P: pump; I: injector; V: switching valve; M: mixer; Heat: heating oven at 56°C; D: fluorescence detector. Column Carbon: BTR carbon, 50×4.6 mm I.D., 3.5 μm; C_{18}: TSK super ODS, 50×4.6 mm I.D., 2 μm; Eluent: 10 m*M* sodium citrate pH 4.5 containing 5 m*M* sodium octanesulfonate, flow rate: 1 ml/min; Nin: aqueous ninhydrin solution (6 g/l), flow rate: 0.2 ml/min; NaOH: 1 *M* sodium hydroxide solution, flow rate: 0.2 ml/min.

octadecyl-bonded silica gel columns. Therefore, ion-pair liquid chromatography was performed using octyl- and octadecyl-bonded silica gel and graphitic carbon columns.

The ion-pair reagent effect was different in these columns. Longer the alkyl-chain length strengthened the retention of paired-ions on the octadecyl-bonded silica gel. The retention factors of ion-paired guanidino compounds were large in the graphitic carbon column, and some retention times were over 4 h. However, the maximum retention factors were obtained using nonanesulfonate on the graphitic carbon column. The difference may have been due to the smoothness of the surface of packing materials. The surface of graphitic carbon was flat and smooth, and showed no selective retention of ion-pair reagents of different sizes. The surface of the bonded silica gel, however, showed selective retention of longer alkyl groups.

Increasing the concentration of ion-pair reagents, increased their retention times in both systems except for very polar compounds such as GSA, TAU and CT. This phenomenon should be due to the exclusion effect at higher concentrations of ion-pair reagents on the graphitic carbon columns. This may be due to the limitation of surface area.

The most suitable eluent consisted of 10 m*M* sodium citrate (pH 4.5 adjusted with 10 m*M* citric acid) containing 5 m*M* sodium 1-octanesulfonate. MG, GPA, CTN, G, ARG and GBA were separated on an octadecyl-bonded silica gel column, and GAA, GSA, TAU and CT were separated on a graphitic carbon column. A chromatogram of standard compounds is shown in Fig. 5A. In this system, L-

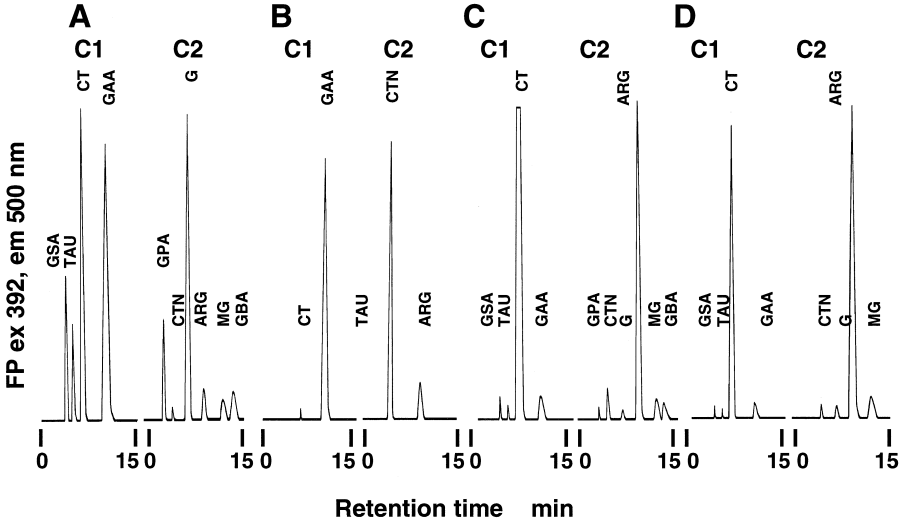

Fig. 5. Chromatograms of guanidino compounds in sera from nephritic patients. Sample A, standard; B, normal subject; C, CGN; D, DMN; Chromatographic conditions: see Fig. 4.

arginine which is a physiological precursor of nitric oxide [66] could be analyzed as a guanidino compound.

The sensitivity of these guanidino compounds was 7.8 ng/ml and the calibration curves were linear (r^2=0.999) from 10 to 2000 ng/ml, except creatinine whose sensitivity was 10-fold less than those of the others. The amount injected was 50 ng except for creatinine (500 ng). The recovery was more than 90%, and the standard deviation was less than 3% [53].

2.2. Analysis of serum

The total number of blood samples was 185 including 20 from patients not treated using hemodialysis and 10 from normal subjects. The total number of doners was 109, ranging in age from 22 to 88 years old including 41 females and 68 males. Seventy-six samples were collected before and after hemodialysis. Blood samples collected were centrifuged at 1000 *g* for 15 min at room temperature, and the sera were stored at −30°C. Examples of chromatograms of sera from a normal subject and different nephritic patients are shown in Fig. 5B–D, respectively. The values of CTN, blood urea nitrogen (BUN) and uric acid (UA) were measured by routine clinical methods for the further discussion of liquid chromatographic data.

The contents of guanidino compounds varied significantly between subjects, and the individual values could not be used for diagnostic purposes. G, GPA, MG, GBA, GSA and TAU were not found in sera from normal subjects. GBA was found in sera of only two of 96 patients, but these sera did not contain significantly large amounts of other guanidino compounds. In one serum sample from a CGN patient, the contents of G, GPA, MG, GBA, GSA, GAA, TAU, CT, ARG and CTN were 0.22, 1.24, 4.46, 3.02, 10.72, 1.20, 0.27, 607.25, 97.14 and 985.78 μmol/l, and those in another serum sample from a patient with DMN were 0.42, 0.0, 1.54, 0.0, 7.03, 2.10, 0.67, 53.43, 97.30 and 1126.90 μmol/l, respectively. The chromatogram of the former serum sample is shown in Fig. 5C, and that of the latter is shown in Fig. 5D. TAU was not common in the sera of these patients but was often found in sera of CGN patients. G, GPA, MG, GSA, TAU and CT were present at higher levels in sera from female subjects, and CTN, BUN and UA levels were higher in those from males [53].

The effects of hemodialysis were analyzed from the contents of guanidino compounds. GPA, GBA and TAU could be effectively removed during hemodialysis, but G, MG, CT and ARG could not be eliminated by hemodialysis. The removal rate of MG was about 48% in CGN and 37% in DMN. The poor removal rate of MG might have been brought about by its immediate synthesis from CTN by the effect of active oxygen. There were no sex-related differences in the removal rate guanidino compounds. The efficiency of the elimination of guanidino compounds varied between subjects. The further analysis of the data after standardization of the individual concentrations using the CTN value indicated the possibility of identifying a disease from the differences in the concentration ratios between guanidino compounds measured in different groups. The ratio of ARG/CTN was high in normal subjects; however that was low in the sera of patients being treated using hemodialysis due to the poor exclusion of CTN from patients. In the sera of the patients with sclerotic kidney (SK), the ratios of ARG/CT, ARG/CTN and GAA/CTN were high, as they were in the normal subjects even though the amount of CTN was highly concentrated. The ratio of CT/MG and ARG/GSA were also high, and those of GAA/ARG, CTN/CT and MG/G were low among patients. In the sera of the diabetic mellitus nephropathy (DMN) patients not being treated with hemodialysis, the ratios of CTN/CT, ARG/CTN and CT/MG were high and those of CT/GAA, CTN/CT and MG/G were low compared with those in the sera of the patients being treated with hemodialysis. The comparison of these ratios in the sera of DMN and CGN patients being treated with hemodialysis indicated the difference of metabolism in the two groups. The ratios of GAA/CTN, CT/MG and ARG/GPA were high and those of GAA/GPA and MG/G were low in the DMN patients.

If these guanidino compounds followed the ordinary metabolic pathway shown in Fig. 1, the correlation coefficient between the amounts of precursors and those of metabolites would have been higher. Therefore, the metabolic conditions were

studied by analyzing the correlation coefficients between pairs of several metabolites.

The correlation coefficient between ARG and CT was 0.997 ($n=10$) for normal subjects, and those between ARG and GAA, and GAA and CT were 0.853 and 0.835, respectively. These results indicated that CT reflects the amount of ARG in serum. However, such high correlation coefficients were not obtained from the sera of the nephritic patients being treated by hemodialysis. The high correlation between ARG and GAA was also obtained except SK patients. The high correlation between GAA and CT was obtained only in the sera of DMN patients (no hemodialysis (noHD) and post hemodialysis (postHD)). Acceptable correlation coefficients were obtained based on the contents of MG and G, ARG and G for CGN patients being treated with hemodialysis, and ARG and CT, GAA and CT, for DMN patients being treated by hemodialysis. The pattern recognition of different diseases is demonstrated in Fig. 6 using several correlation coefficients (r). SK, DMN without hemodialysis, DMN with hemodialysis and CGN with hemodialysis demonstrated individual patterns. This type analysis will help to diagnose diseases; however more samples of different diseases are necessary to improve the precision of the diagnoses.

The comparison of the concentrations of CT, CTN and MG and their correlation values indicated the precursor of MG in DMN and CGN. The concentration of CTN was high in sera from CGN, therefore that of MG was high too in sera from CGN patients. However, the correlation between MG and CTN was very high, 0.868, in the sera from the DMN patients, and the correlation value was 0.501 from the CGN patients. The correlation between MG and CT was 0.594 in the sera from the CGN patients and 0.222 in the sera from the DMN patients. These results suggest that MG was readily synthesized from CTN where active oxygen involved in the synthesis reaction [67–73], and that MG was synthesized from CT [74]. The results also suggested that the synthesis pathways are different among the diseases studied. MG was mainly synthesized from CTN in DMN, and MG was synthesized from both CT and CTN in CGN. The correlation between GSA and CTN was 0.654, and that between ARG and GSA was zero in the sera from DMN patients being treated by hemodialysis. Such high correlation coefficients were not observed in the other disease groups. These results indicated that a different synthesis pathway to GSA from CTN may exist.

In addition, the contents of guanidino compounds in sera from selected patients (10 DMN and 10 CGN

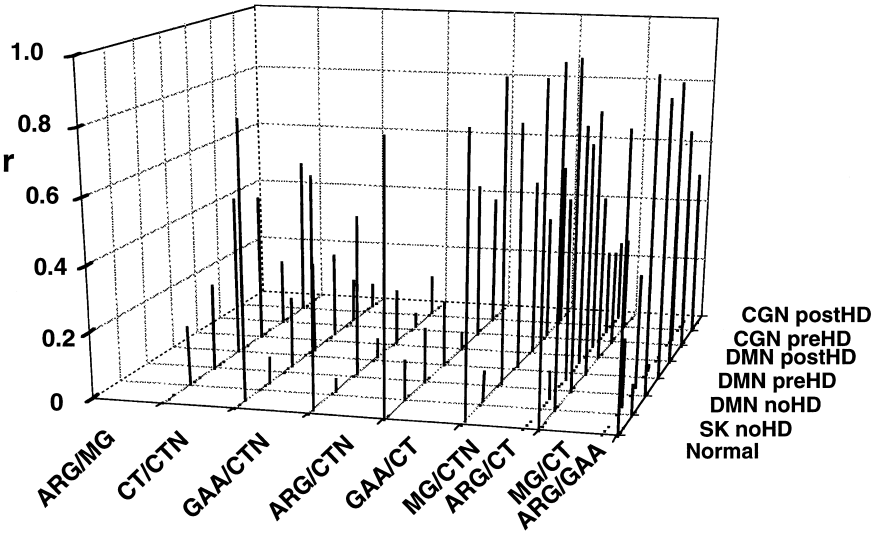

Fig. 6. Content ratios of guanidino compounds found in sera from different patients before and after hemodialysis. CGN: chronic glomerulonephritis, DMN: diabetic nephropathy, SK: sclerotic kidney, HD: hemodialysis.

patients) were not changed after 6 months of hemodialysis (except for one patient). The removability of the compounds also remained the same. This means that the interval of hemodialysis can be expanded if the amount of guanidino compounds was concerned as the important indicators for hemodialysis.

2.3. Diagnosis purpose

The liquid chromatograph equipped with a graphitic carbon column and a post-column labeling detector was reproducible system to analyze a trace level of guanidino compounds in human sera as a routine analytical method due to the chemical and physical stability of the graphitic carbon column and the sensitivity of detector. Furthermore, a liquid chromatographic system consisting of two selective columns and a single eluent permitted the baseline separation of ten guanidino compounds in serum within 15 min. The newly developed liquid chromatographic system for the analysis of guanidino compounds was stable for the long term operation, over 6 months, and rapid enough for the routine (clinical) analysis of ten guanidino compounds in serum. This system can handle 96 serum samples per day. This system was applied to monitor hemodialysis and for the analysis of different types of nephritic. The efficiency of hemodialysis seemed to be related to differences between individual subjects not the properties of guanidino compounds. The contents of guanidino compounds in sera from patients with chronic glomerulonephritis were larger than those from subjects with diabetic mellitum nephropathy. The correlation between the concentration of creatine, creatinine and methylguanidine made clear the synthesis process of methylguanidine in different diseases. Methylguanidine was mainly synthesized from creatinine in diabetic mellitum nephropathy, and that was synthesized from both creatine and creatinine in chronic glomerulonephritis. Guanidinosuccinic acid may be synthesized from creatinine in diabetic mellitum nephropathy due to the acceptable correlation between guanidinosuccinic acid/creatinine but not arginine/guanidinosuccinic acid which is believed as the major metabolic pathway. Above results indicates that the further analysis of guanidino compounds in serum can improve the diagnosis of diabetics.

3. Arginine and arginine derivatives for nitric oxide synthesis

The measurement of L-arginine, a physiological precursor of nitric oxide, was used in studies of the disposition of exogenous doses of L-arginine [66]. The L-arginine-nitric oxide system regulates platelet aggregation in pregnancy [75]. Chronic blockade of nitric oxide synthesis elevated plasma levels of catecholamines and their metabolites at rest and during stress in rats [76]. Serum nitrite sensitivity reflected endothelial nitric oxide formation in human forearm vasculation [77]. Assessment of nitric oxide production was measured by [^{15}N]citrulline in human plasma by LC–MS [78,79]. Changes in plasma L-arginine levels were observed in spontaneously hypertensive rats under hypotension [80]. The pharmacokinetics of L-arginine during chronic administration to patients with hypercholesterolemia have been studied [81].

Several arginine derivatives are inhibitors of nitric oxide synthase isoenzyme which catalyzes the formation of nitric oxide from the L-arginine. Tsikas et al. studied interference of the nitric oxide synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-nitro-L-arginine (L-NNA). They measured nitrate in urine as a reliable approach to assess NO formation in vivo by chemiluminescence and gas chromatography–mass spectrometry [82]. The distribution and metabolism of L-NAME in patients with septic shock was analyzed by Avontuur et al. [83]. L-NNA reduced tolerance to morphine in the rat locus coeruleus [84].

Tsikas et al. analyzed *N,N*-dimethylarginine (DMA), NG,NG-dimethylarginine (ADMA) and N^G,N^G-dimethyl-L-arginine (SDMA) in human plasma using liquid chromatography all of which are endogenous compounds occurring in several tissue proteins, plasma and urine of humans, and ADMA is a potent inhibitor of nitric oxide synthesis [85]. Asymmetric DMA plasma concentration differed in patients with end-stage renal diseases [86]. SDMA in plasma of dialysis patients was measured by Anderstam et al. [87]. N^G-momomethyl-L-arginine is a

competitive inhibitor of nitric oxide synthesase isoenzymes that catalyze the formation of NO from L-arginine in a variety of cell types. N^G-momomethyl-L-arginine was analyzed using liquid chromatography after pre-column derivatization with *o*-phthalaldehyde [88,89]. N^G,N^G-dimethylarginine in human plasma was also analyzed by liquid chromatography [90–92]. *N*(omega)-Allyl-L-arginine is a competitive reversible inhibitor and time-dependent inactivator of bovine brain nitric oxide synthase [93]. Feng et al. determined the levels of agmatine, decarboxylated arginine, a candidate neurotransmitter that may bind to the imidazoline receptor in plasma and brain by liquid chromatography [94].

4. Pharmaceutical compounds having a guanidino group

A variety of compounds having a guanidino group are pharmaceutically important drugs. These include clonidine hydrochloride, guanabenz acetate, guanethidine sulfate, bethanidine sulfate, guanfacine hydrochoride, arginine glutamate, nafamostat mesilate, gebexate mesilate, camostat mesilate, metformin hydrochloride and buformin hydrochloride.

Other drugs are aminoglycoside antibiotics and peptides containing arginine. These are not related to guanidino compounds which are natural metabolites of arginine. However, someone interests in their chromatographic condition. The date are summarized in Table 1.

5. Nomenclature

ACE	angiotensin I-converting enzyme
ADMA	N^G,N^G-dimethylarginine
APCI	atmospheric pressure chemical ionization
ARG	arginine
BUN	blood urea nitrogen
CAPD-RT	continuous ambulatory peritoneal dialysis after renal transplantation
CGN	chronic glomerulonephritis
CT	creatine
CTN	creatinine
DMA	*N,N*-dimethylarginine
DMN	diabetic nephropathy
FGS	focal glomerular sclerosis
G	guanidine
GAA	guanidinoacetic acid
GBA	γ-guanidinobutyric acid
GK	gouty kidney
GPA	β-guanidinopropionic acid
GRH	gonadotrophin-releasing hormone
GSA	guanidinosuccinic acid
h-EGF	human epidermal growth factor
h-GH	human growth hormone
HT	hypertension
IgAGN	IgA glomerulonephritis
LC	liquid chromatography
L-NAME	N^G_G-nitro-L-arginine methyl ester
L-NNA	N^G-nitro-L-arginine
MG	methylguanidine
MN	membranous nephropathy
MPGN	membraneproliferative glomerulonephritis
MS	mass spectrometry
NS	nephrotic syndrome
PCR	polymerase chain reaction
SDMA	N^G,N^G-dimethyl-L-arginine
SK	sclerotic kidney
TAU	taurocyamine
UA	uric acid

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