Automatic System for the Assay of Guanidino Compounds to Assess Uremic Status and Effect of Hemodialysis

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An automated HPLC system coupled with fluorometry was established for the sensitive, rapid, and accurate assay of serum guanidines. Naturally fluorescent materials characteristic of the sera of uremic patients (uremic fluorescences) which interfere with the assay were removed simultaneously with deproteinization. Application of this method revealed that the uremic patients who are capable of excreting urine under hemodialysis therapy show low serum guanidinosuccinic acid levels. The interval between hemodialysis sessions in one of these patients was prolonged while monitoring guanidinosuccinic acid level using the present method without any hazardous effect.

Key words guanidinosuccinic acid; methylguanidine; hemodialysis; HPLC; uremia; anuria; automated analysis.

Serum creatinine (CTN) and blood urea nitrogen (BUN) have been used as diagnostic parameters of renal dysfunction. They are referred to in deciding when to introduce patients to hemodialysis and for evaluating the effect of hemodialysis. However, introduction to hemodialysis should be decided based on clinical symptoms rather than these values, they are not effectively removed in hemodialysis, and are regarded as only approximate indices.

Guanidines have been regarded as candidates[1,2] for the index of renal dysfunction since they are more toxic than CTN or BUN. Various methods for the determination of guanidines using HPLC coupled with fluorometry have been developed[3—6]. Conventional HPLC methods were, however, interfered with by uremic fluorescences in the sera of uremic patients and their removal was tedious and time-consuming. Moreover, these methods gave preference to the resolution of all guanidines and separation required skill.

The present method therefore focuses on the estimation of guanidinosuccinic acid (GSA) and methylguanidine (MG), which have toxicity and seem to reflect the actual pathological state. The procedure including deproteinization is fully automated. In addition, uremic fluorescences are removed together with serum proteins using an exclusion column with hydrophobic pores, coupled with the column-switching technique.

Figure 1 shows the flow diagram of the present automated HPLC system controlled by a host computer (Model Vectra XM series 3 16/90, Yokogawa Analytical Systems Inc., Tokyo, Japan). Eluent 1 contained 5 mM ninhydrin in 100 mM phosphate buffer (pH 7.0). Eluent 2 contained acetonitrile and 0.1% aqueous triethylamine (90 : 10 v/v%). Eluent 3 contained 5 mM ninhydrin, 20 mM 1-octanesulfonic acid sodium salt in a mixture of 10 mM sodium dihydrogenphosphate solution and acetonitrile (85 : 15, pH 4.6). Eluent 4 was 50% aqueous acetonitrile.

Eluent 1 was delivered from pump A (Model G1310A Iso Pump, Yokogawa) at a flow rate of 1.0 ml/min. An aliquot of 50 μl of serum was mixed with 50 μl of dimethylguanidine solution (5 μM) as an internal standard in the autosampler (Model G1313A ALS, Yokogawa), and injected into the precolumn via the line filter (4.6 mm i.d.) and the guard column 1 (Shim-Pak SPC-RP3, 30×4 mm i.d., particle size 9 μm) from the autosampler (0 min). The guanidine fraction was separated from uremic fluorescences and proteins simultaneously on the precolumn (Asahipak GF-310 HQ, 300×7.6 mm i.d.) by eluent 1. The eluent was switched from 1 to 2 using step gradient unit 1 (Model SGR-1A, Shimadzu Seisakusho Co., Kyoto, Japan) 5 min after the sample injection to wash the precolumn for 5 min, and again switched to eluent 1 in order to prepare for the next analysis. The guanidine fraction was transferred to the separation column (Senshu Pak PEGASIL ODS, 150×4.6 mm i.d., particle size 5 μm) *via* guard column 2 (Senshu Pak: PEGASIL ODS, 10×4.6 mm i.d., particle size 5 μm) 7 min after injection using the microelectric two-position valve actuator for column switching (Model EHMA, Valco Instruments Co. Inc., Texas, U.S.A.). The valve was then turned to deliver eluent 3 from pump B (Model G1310A Iso Pump, Yokogawa) at a flow rate of 1.0 ml/min to the separation column on which guanidines formed ion pairs with 1-octanesulfonic acid and were separated. The eluent was then switched from 3 to 4 using gradient unit 2 (Model FCV-9AL, Shimadzu) 22 min after the sample injection to wash the separation column for 5 min, and again switched to eluent 3 to prepare for the next analysis. The effluent containing ninhydrin from the separation column was mixed with 1.0 M sodium hydroxide delivered from the pump C (Model LC-9A, Shimadzu) at a flow rate of 0.5 ml/min. The mixture was heated in a stainless steel coil (5 m×0.5 mm i. d.), placed in a oven at 90 °C, and the fluorescence was monitored at Ex. 395 nm and Em. 500 nm with a spectrofluorometric detector (Model RF-535, Shimadzu). The analog signal from the detector was converted to digital data by an A/D converter (Model HP 35900E Dual Channel Interface, Yokogawa), and the data were transmitted to the host computer to be stored and processed using Chem Station (Yokogawa) chromatographic software.

GSA (Rt. 9 min) and MG (Rt. 22 min) showed linear responses in the range of 50—2500 pmol/injection and 25—

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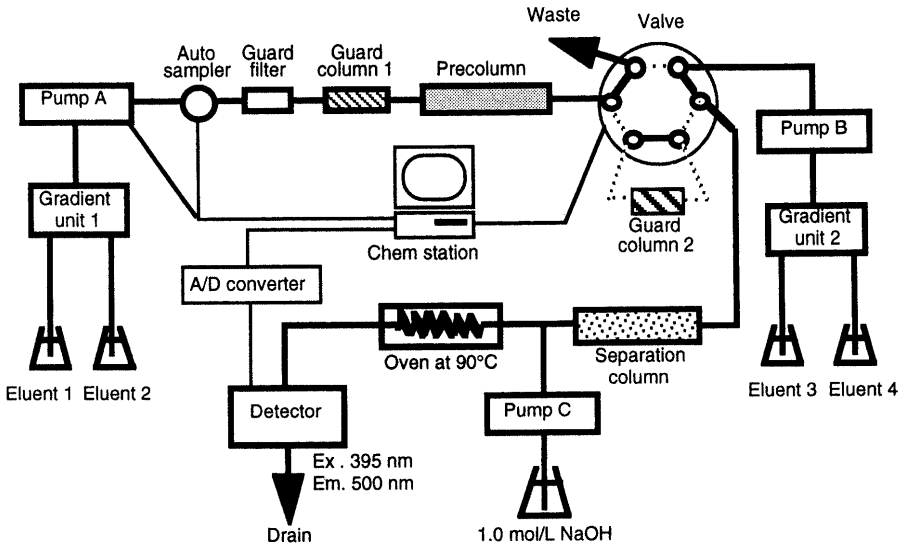

Fig. 1. Automated System for HPLC-Fluorometric Assay of GC

Table 1. Serum Concentration of Uremic Indexes of the Patients Capable of Excreting Urine after 7 and 9 d from the Last Hemodialysis in Comparison with Those of Anuric Patients (n=23) Just before the Hemodialysis

	Patient 1	Patient 2		Anuric patients
	7 d	7 d	9 d	(mean±S.D.)
GSA (μM)	3.55	8.22	9.02	28.47±13.24
MG (μM)	2.99	2.71	2.93	4.79± 2.62
CTM (mM)	0.52	0.76	0.74	1.21± 0.19
BUN (mM)	8.81	14.29	16.40	13.47± 2.65
MG/CTN	5.75	3.57	3.96	3.96± 1.29

1250 pmol/injection, respectively. The respective coefficients of variation (within-run, n=9) for GSA and MG were 1.8% and 2.0% for the standard solution of 1000 and 500 pmol of samples. The detection limits of GSA and MG were 50 and 25 pmol (S/N=2), and the recoveries from human serum were 96.9% and 99.4%, respectively.

BUN has so far been used as the index for the efficiency of hemodialysis since it easily passes through the cell membrane and can be removed readily from the biological pool as well as from blood. Although GSA was regarded as a better index, because its removal rate was better than BUN, uremic fluorescences overlapped with GSA to affect the assay. The present method allows automatic removal of uremic fluorescences and provides ready and precise determination of GSA to give reliable data for the evaluation of hemodialysis therapy.

The present method was then applied to the analysis of GSA and MG in the sera and urine of uremic patients capable of urine excretion under hemodialysis. Uremic patients ordinarily develop anuria on introduction to hemodialysis therapy. On the other hand, chronic glomerulonephritic patients with sufficient renal blood flow can be introduced to hemodialysis without causing anuria with the proper oral supply of water. Although the prevention of anuria contributes greatly to the quality of life (QOL) of patients, its clinical significance remains unclear since the conventional indexes such as CTN and BUN show little distinct difference between anuric patients and patients capable of urine excretion.

Table 1 shows that uremic patients (patients 1 and 2) capable of urine excretion under hemodialysis have far lower serum concentrations of GSA than anuric patients. MG and CTN were also lower in patients 1 and 2 although the difference was not significant. The BUN and MG/CTN ratio, which is an index of active oxygen, showed little difference between anuric patients and patients excreting urine.

Prolongation of the interval between hemodialysis session was then attempted. The interval of hemodialysis therapy for patient 2, who was hemodialysed every 7 d, was prolonged by 2 d. Table 1 shows that none of the compounds estimated in the sera of patients excreting urine changed significantly due to prolongation of the interval.

The present study confirmed that the urine excretion of uremic patients is important for the improvement of their pathological state. Moreover, prolongation of the interval between hemodialysis sessions may be safer with the monitoring of GSA together with MG and CTN, and it should greatly contribute to the promotion of patient QOL. The present system is suitable for this purpose and expected to be widely used in the clinical field.

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