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Creatol, an Oxidative Product of Creatinine in Hemodialysis Patients

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Creatol (CTL) is a product resulting from the reaction of creatinine (Cr) with the hydroxyl radical and is identified as a precursor of methylguanidine (MG), a uremic toxin. In this study, we investigated serum CTL levels together with those of Cr and MG in 66 patients who were on maintenance hemodialysis (HD). Prior to dialysis, the mean serum levels of Cr, CTL and MG were 967 (= 11.1 mg/dl) ± 267 μM, 11.1 ± 4.8 μM and 5.8 ± 2.9 μM, respectively. The mean CTL level was about 1.1% that of Cr, and the CTL plus MG level was about 1.4% that of the Cr level. The reduction rates of Cr, CTL and MG by a single HD were 62.6 ± 6.1%, 71.0 ± 10.3% and 51.9 ± 11.6%, respectively. The CTL level at 0.5, 1 and 6 h after HD increased rapidly by 20.7 ± 8.7%, 31.7 ± 14.7% and 80.1 ± 27.3%, respectively. There was a significant correlation between CTL or CTL/Cr and parathyroid hormone in patients who had just undergone parathyroidectomy. No significant correlation was found between CTL or CTL/Cr and those factors which seems to be related to the predialysis levels of reactive oxygen. Therefore, because of the good clearance of CTL and its rapid conversion to MG, its usefulness for the estimation of hydroxyl radical generation in HD patients is limited.

Keywords: Creatol, methylguanidine, hydroxyl radical, hemodialysis

INTRODUCTION

Recently, creatol (CTL) was identified as a product of the reaction of creatinine (Cr) with the hydroxyl radical. CTL further converts to creaton A, creaton B, and then methylguanidine (MG).[1,2] MG is a toxic compound and its synthesis increases in uremic patients.[3–5] Recently, MG was reported to be an inhibitor of nitric oxide synthesis.[6] We, in turn, have reported that MG is synthesized from Cr via the hydroxyl radical[7] and, therefore, proposed that the molar ratio of MG/Cr is a useful marker of hydroxyl radical generation under some conditions.[8–10] High concentration of serum MG and increased MG/Cr ratio were reported in patients with secondary

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hyperparathyroidism or dialysis-related amyloidosis.[11,12] In hemodialysis (HD) patients the MG/Cr ratio showed a significant correlation with N^{ε}-(carboxymethyl)lysine, which may contribute to dialysis-related amyloidosis.[13] Therefore, a study of CTL in HD patients was undertaken to evaluate this Cr metabolite as a marker for the hydroxyl radical and, also, to examine Cr metabolism in HD patients.

In this study, we assayed serum CTL together with Cr and MG following a single HD to determine the clearance of CTL, its variation after HD and its relation to factors which seem to affect the generation of hydroxyl radical in HD patients.

SUBJECTS AND METHODS

Patients

All studies were performed after the 68th hour following the last HD. Serum Cr (s-Cr), CTL (s-CTL), MG (s-MG) and urea nitrogen (BUN) concentrations were examined in 66 HD patients (34 males, 32 females) at the beginning and the end of HD. The age was 63 ± 13 (mean $\pm$ SD, range 25–84) years and the duration of HD treatment was 7.3 ± 6.5 (0.5–22) years. The time for a single HD session was 240 ± 12 min. The blood flow rate was 180 ± 26 ml/min and the dialysate flow rate was 500 ml/min. Synthetic dialyzers were used in 57 patients and cellulosic dialyzers in 9 patients with a mean surface area of $1.6 \pm 0.3\,m^2$. A bicarbonate-based dialysate was used in all patients. Origins of the end stage renal disease (ESRD) in the group were: chronic glomerulonephritis ($n = 28$), diabetic nephropathy ($n = 13$), polycystic kidney disease ($n = 4$), nephrosclerosis ($n = 2$), lupus nephritis ($n = 2$), tubulointerstitial nephritis ($n = 1$), rapidly progressive glomerulonephritis ($n = 1$), renal tuberculosis ($n = 1$), scleroderma kidney ($n = 1$), pregnant hypertension ($n = 1$) and unknown ($n = 12$).

In an additional 4 patients, the levels of s-Cr, s-CTL, s-MG and BUN were examined at the beginning of HD, at 1, 2, 3 and 4 h after initiation of HD and 0.5, 1, 6 and 44 h following the end of HD. Case 1 was a female, aged 58, who underwent bilateral nephrectomy for urethral cancer and had received maintenance HD for 1 year. Case 2 was a female, aged 51, treated with HD for 1 year after 2 years peritoneal dialysis for ESRD due to chronic pyelonephritis. She had just undergone the parathyroidectomy 5 days before this study for severe secondary hyperparathyroidism. Case 3 was a female, aged 56, who had received HD for 10 years for ESRD due to chronic glomerulonephritis. Case 4 was a female, aged 52, who had started maintenance HD 2 months before for uremia due to diabetic nephropathy. She had a urine output of 600 ml per day.

Sample Collection

Blood samples were collected from the arterial side of the arteriovenous fistula during HD. After HD, samples were collected from the brachial vein on the side opposite the dialysis arm.

Analytical Method for Cr, CTL, MG and Blood Urea Nitrogen

Cr and BUN were determined by an autoanalyzer using an enzymatic method. Before analysis for CTL and MG, all sera were kept under −20°C, following which they were deproteinized with trichloroacetic acid (final concentration: 10% w/v). After centrifugation at 1500*g* for 15 min, the supernatants were used for CTL and MG determinations. MG was determined by HPLC analysis using 9,10-phenanthrenequinone (PQ) for post-labeling as described previously.[14] CTL, separated using a cation-exchange resin was converted quantitatively to MG by strong alkali and high temperature, and was determined by HPLC using a PQ reaction.[15,16]

Calculation

The reduction rate and the rebound rate were calculated using the following formulae;

$$\text{Reduction Rate} = (C1 - C2)/C1 \times 100\,(\%)$$
$$\text{Rebound Rate} = (CR - C2)/C2 \times 100\,(\%)$$

C1: concentration at the beginning of HD.
C2: concentration at the end of HD.
CR: concentration at each time after HD.

Statistics

Data are presented as the mean ± SD. Correlation coefficients were calculated by linear regression analysis. Statistical analysis was performed by the Mann–Whitney test, and statistical significance levels were corrected by the Bonferroni/Dunn's equation. A p value less than 0.05 was considered significant.

RESULTS

Chromatogram of CTL

A typical chromatogram showing serum CTL in a HD patient before and after HD compared to the CTL standard is shown in Figure 1.

Variation of s-CTL Following a Single HD

The levels of s-Cr, s-CTL and s-MG were determined before and after HD as shown in Table I. The mean levels of s-CTL and s-CTL plus s-MG before HD were respectively 1.1% and 1.7% that of s-Cr. The mean level of s-CTL was about 1.9 times that of s-MG.

The reduction rates of s-Cr, s-CTL and s-MG during a single HD session are also shown in Table I. s-CTL was reduced most of all by a single HD and its reduction rate was higher than that of s-MG by 39%.

Statistical Analysis of the Serum Level of CTL

Before HD, a significant correlation was found between the concentration of s-Cr and s-CTL

TABLE I Concentrations of BUN, Cr, CTL and MG before and after HD, and their reduction rates by a single HD

	BUN (mg/dl)	Cr (μM)	CTL (μM)	MG (μM)
Before HD	67.0 ± 17.0	967 ± 267	11.1 ± 4.8*	5.8 ± 2.9*
After HD	21.0 ± 8.0	362 ± 118	3.1 ± 1.6*	2.8 ± 1.6*
Reduction rate (%)	68.7 ± 7.4	62.6 ± 6.1	71.0 ± 10.3*	51.9 ± 11.6*

Values are expressed as mean ± SD. Statistical significance: *$p < 0.0001$ vs Cr.

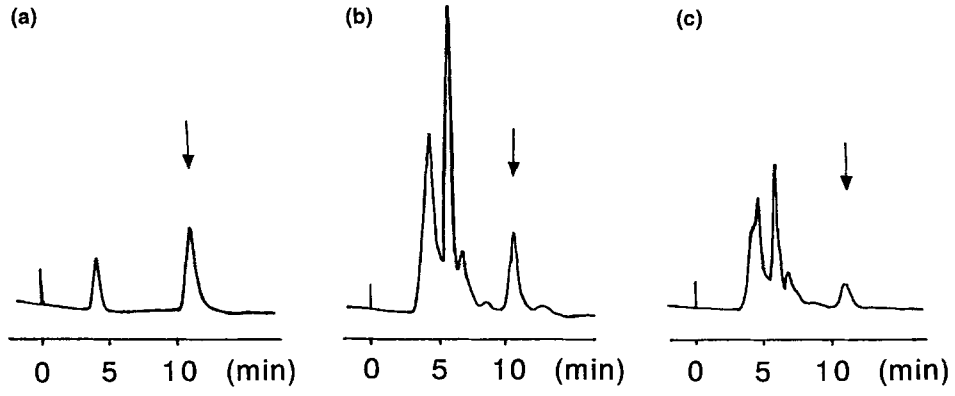

FIGURE 1 A chromatogram of CTL is shown. The arrow shows the peak of CTL. A standard peak is shown in (a), reflecting a 2.0 μM concentration of CTL. The serum from a patient before HD is shown in (b) and serum from a patient after HD is shown in (c).

($r = 0.602$, $p < 0.0001$) (Figure 2(a)), and between the concentration of s-Cr and s-MG ($r = 0.749$, $p < 0.0001$) (Figure 2(b)). The correlation between s-Cr and s-MG was greater than that between s-Cr and s-CTL. After HD, the correlation between s-Cr and s-CTL was weaker than that before HD.

Before HD, the correlation between s-Cr and the molar ratio of MG/Cr (mean ± SD, 0.0058 ± 0.0021) was significant ($r = 0.365$, $p = 0.0026$) (Figure 2(d)). However, the correlation between s-Cr and the molar ratio of CTL/Cr (0.0116 ± 0.0044) was not significant ($r = -0.026$, $p = 0.8362$) (Figure 2(c)).

Time Course of Serum CTL During and After HD

The changes in the concentrations of s-Cr, s-CTL, s-MG and BUN during and after a single HD are shown in Figure 3(a) (case 1), (b) (case 2), (c) (case 3) and (d) (case 4). Case 2 who had just undergone parathyroidectomy showed

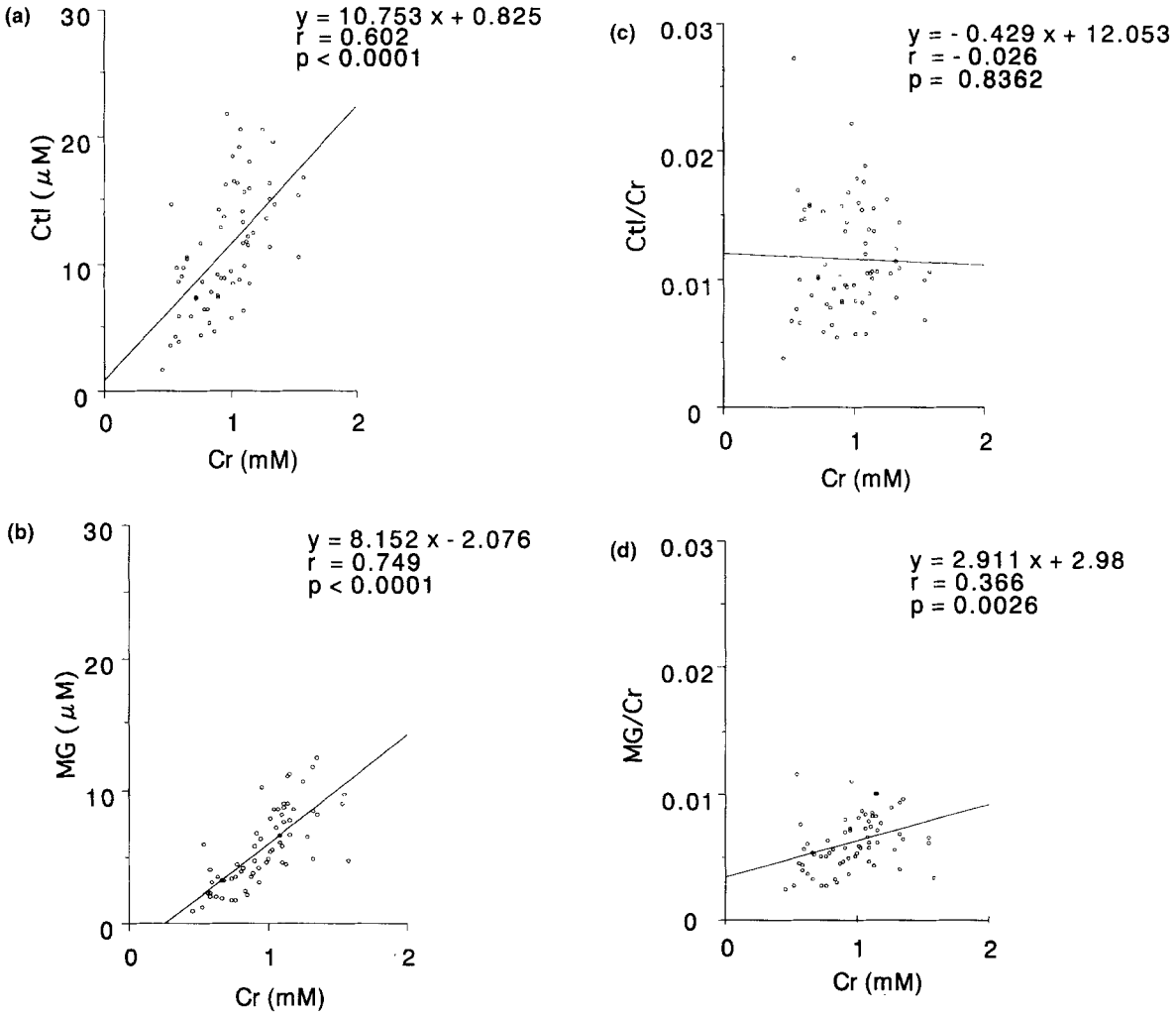

FIGURE 2 Correlations between s-Cr and s-CTL, s-MG, CTL/Cr and MG/Cr are shown. Individual data are shown by open circles. Each of the coefficients of correlation are shown at upper right side of each figure.

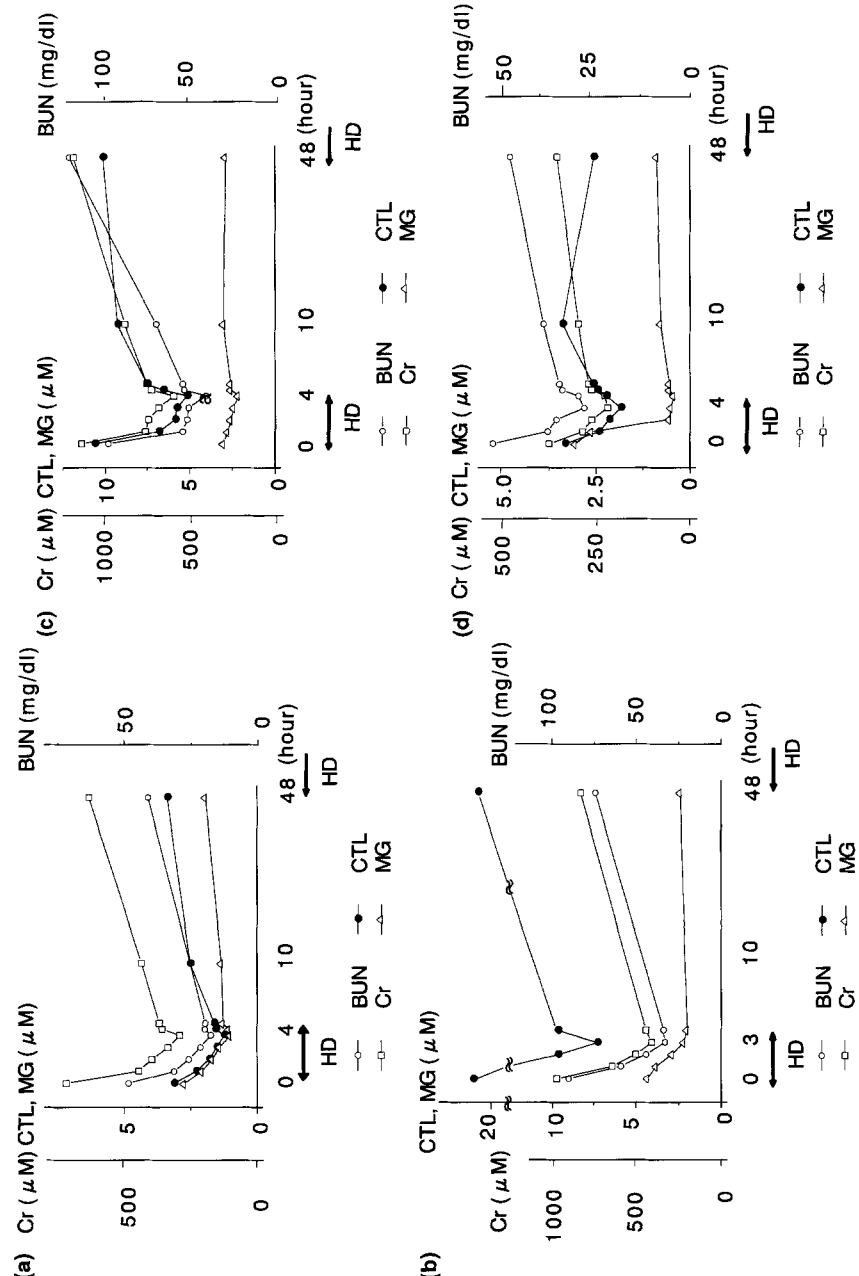

FIGURE 3 Changes in the serum concentrations of BUN, Cr, CTL and MG throughout and after HD. The concentrations of BUN are shown by open circles (○), the concentrations of Cr are shown by open squares (□), the concentrations of CTL are shown by solid circles (●) and the concentrations of MG are shown by open triangle (△). Each concentration was determined at the beginning of HD, at 1, 2, 3 and 4 h from the beginning, and at 0.5, 1, 6 and 44 h following the end of HD.

significantly higher concentrations of s-CTL and CTL/Cr compared with the other 66 cases ($p < 0.0001$). The rebound rates of BUN, s-Cr, s-CTL and s-MG at 0.5, 1 and 6 h after HD are shown in Table II. The increase in the rate of s-CTL was the highest of all at every time period examined, and especially at 6 h after HD, when it increased about 80% from that at the end of HD.

Statistical Analysis of the Correlation between CTL and Factors which Seem to be Related to the Hydroxyl Radical

S-Cr, s-MG and the molar ratio of MG/Cr were significantly correlated with β_2-microglobulin (β_2-MG) ($p < 0.05$) in 66 patients prior to HD. There was no correlation between s-CTL or CTL/Cr and β_2-MG or other factors such as hematocrit, high-sensitive parathyroid hormone (HS-PTH), iron or ferritin all of which seem to be related to reactive oxygen.

Patients who had received HD more than 3 years ($n = 46$) showed significantly higher concentrations of s-Cr, s-CTL, s-MG and MG/Cr (Table III). CTL/Cr was also higher in this group but the difference was not significant. Patients who had diabetic nephropathy ($n = 13$) showed significantly lower concentrations of s-Cr ($p < 0.05$) than other patients matched in terms of HD duration ($n = 13$). The s-CTL, s-MG, CTL/Cr and MG/Cr were also lower in diabetic patients but the differences were not significant.

TABLE II Rebound rates of BUN, Cr, CTL and MG at 0.5, 1 and 6 h after HD

	30 min after HD (%)	1 h after HD (%)	6 h after HD (%)
BUN	19.7 ± 11.8	20.9 ± 11.6	47.9 ± 24.9
Cr	20.5 ± 4.4	24.3 ± 4.5	43.4 ± 11.2
CTL	20.7 ± 8.7	31.7 ± 14.7	80.1 ± 27.3
MG	19.0 ± 9.7	26.3 ± 5.9	51.7 ± 31.5

Values are expressed as mean ± SD.

DISCUSSION

This is the first report of serum CTL and its variation in patient undergoing HD.

The mean serum concentrations of CTL and CTL plus MG at 68 h after the last HD are about 1.1% and 1.7% that of Cr. This amount is not negligible in terms of the metabolism of Cr. The reduction rate of CTL during a single HD is higher than that of Cr and much higher than that of MG. The low reduction rate of MG may be explained by its tight binding to serum protein because it is strongly basic. CTL and Cr, on the other hand, are neutral substances and do not bind to serum proteins significantly, so they will have high clearance.

The rebound rate of CTL is higher than that of MG especially at the 1st and 6th hour after the end of HD. S-CTL at the 6th hour after HD increased by about 80% from that at the end of HD, and declined before the next HD. This rapid increasing concentration of CTL may be explained by the rapid transfer of CTL from cells to sera after the end of HD and the slow conversion of

TABLE III Concentrations of Cr, CTL and MG and molar ratio of CTL/Cr, MG/Cr in patients with HD duration more than 3 years or less than 3 years, and with diabetes or non-diabetes are shown

	Cr (μM)	CTL (μM)	MG (μM)	CTL/Cr	MG/Cr
HD duration (> 3 years) ($n = 44$)	1040 ± 245*	12.3 ± 4.5*	6.7 ± 2.8*	0.0121 ± 0.0038	0.0064 ± 0.0028*
HD duration (< 3 years) ($n = 22$)	801 ± 244	8.3 ± 4.3	3.6 ± 1.9	0.0106 ± 0.0054	0.0045 ± 0.0019
Diabetes ($n = 13$)	773 ± 225**	8.5 ± 3.7	4.0 ± 2.4	0.0114 ± 0.0058	0.0050 ± 0.0024
Non-Diabetes ($n = 13$)	1001 ± 272	12.0 ± 6.1	4.9 ± 2.4	0.0116 ± 0.0047	0.0047 ± 0.0013

Values are expressed as mean ± SD.
Statistical significance: *$p < 0.05$ vs HD duration (< 3 years), **$p < 0.05$ vs non-diabetes.

CTL to MG at lower levels of CTL. At a prolonged interval following HD, a equilibrium may develop between intracellular and extracellular concentrations of CTL with CTL in serum converted mostly to MG which explains why the rebound of CTL long after HD was low. The reduction rate of CTL can be overestimated because of its high rebound rate in a short period after HD.

However, the serum concentration of CTL was higher than that of MG and the variation of CTL by a single HD was larger than that of MG. Therefore, CTL will be a more sensitive marker for the hydroxyl radical than MG, which is affected by oxygen stress rapidly in HD patients.

Patients who underwent HD for more than 3 years showed higher levels of CTL/Cr but the results were not significant. Diabetic patients showed lower levels of CTL or CTL/Cr, but these differences were also not significant. Increased serum MG and the MG/Cr ratios in patients with severe hyperparathyroidism or dialysis-related amyloidosis suggest a marked increase of reactive oxygen in these patients. The patient who underwent parathyroidectomy showed a significantly higher concentration of CTL, but there was no correlation between s-CTL and HS-PTH. The reason for the lack of correlation between CTL or CTL/Cr and the factors affecting reactive oxygen in HD patients may be the large variation in CTL produced by a single HD.

In conclusion, CTL will be a more sensitive marker of hydroxyl radical than MG, which is rapidly affected by oxygen stress in HD patients.

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