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Efficient Removal of Albumin-Bound Furancarboxylic Acid, an Inhibitor of Erythropoiesis, by Continuous Ambulatory Peritoneal Dialysis

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Key Words. Continuous ambulatory peritoneal dialysis · Furancarboxylic acid · Albumin binding · Inhibitor of erythropoiesis · Uremia · Anemia

Abstract. 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid, which cannot be removed by conventional hemodialysis due to its strong albumin binding, was found to be efficiently removed by continuous ambulatory peritoneal dialysis (CAPD), resulting in a lower serum level in uremic patients on CAPD than in those on hemodialysis. 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid was demonstrated in vitro to inhibit erythroid colony formation. The anemia in patients on CAPD was significantly less severe than in those on hemodialysis. These results suggest that the efficient removal by CAPD of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, an inhibitor of erythropoiesis, is related to an improvement of anemia in patients on CAPD.

Introduction

The albumin-bound metabolites such as indoxyl sulfate and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, which cannot be removed efficiently by conventional hemodialysis, are markedly accumulated in uremic serum as inhibitors of drug binding [1, 2]. To determine if these albumin-bound metabolites can be efficiently removed by continuous ambulatory peritoneal dialysis (CAPD), their serum levels in uremic patients on CAPD were compared with those on hemodialysis, and their removal by CAPD was determined by quantifying their concentrations in equilibrated peritoneal dialysate.

CAPD has been reported to produce an improvement in the anemia associated with end-stage renal disease [3, 4]. Many factors are involved in the anemia of chronic renal failure including erythropoietin deficiency, inhibition of erythropoiesis, and shortened red cell life span. Recent data on the clinical effect of recombinant human erythropoietin for anemia in uremic patients indicate that erythropoietin deficiency is the major mechanism for the anemia of chronic renal failure [5]. However, inhibitors of erythropoiesis were also involved in the anemia of chronic renal failure, since uremic serum inhibited erythropoiesis, and the degree of serum inhibition of erythropoiesis correlated well with the degree of anemia [6]. Spermine, which accumulates in uremic serum, was identified as an inhibitor of erythropoiesis [7]. However, the role of polyamines, spermine, and spermidine in the anemia of chronic renal failure is controversial [8]. The improvement of anemia in CAPD patients is postulated to be related to a more efficient removal of high-molecular-weight uremic toxins which have been considered responsible for the inhibition of erythropoiesis [9]. This study was designed to examine the inhibitory effect on erythropoiesis of albumin-bound metabolites.

Methods

Subjects

Twenty patients (13 males, 7 females) on CAPD, dialyzed for 1.08 years (SD 0.87), ranging in age from 30 to 63 years, and 23 patients (12 males, 8 females) on maintenance hemodialysis, dialyzed for 1.06 years (0.54), ranging from 30- to 70-year-old were selected for the study.

Normal serum samples were collected from 11 healthy subjects (5 males, 6 females). Normal urine samples were collected from 20 healthy subjects (10 males, 10 females). All the samples were stored at −20 °C until they were analyzed.

Chemicals

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid was kindly supplied by Prof. G. Spiteller, Bayreuth University, FRG. Indoxyl sulfate potassium salt was obtained from Nakarai Chemical Co., Kyoto, Japan. Methylcellulose was obtained from Wako Pure Chemical Co., Osaka, Japan. Bovine serum albumin was purchased from Sigma Chemical Co., St. Louis, Mo., USA. Erythropoietin was obtained from Toyobo, Osaka, Japan. All the other chemicals used were of analytical grade.

Sample Preparation for High-Performance Liquid Chromatography

To quantitate total 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, deproteinization of serum and dialysate was performed according to the method of Mabuchi nd Nakahashi [10] by boiling at 100 °C for 5 min. The gelatinous sample was triturated with microspatula and then centrifuged for 30 min at 1,000 *g* to remove protein. The supernate filtered through a 0.45-μm Chromatodisc 13 A filter (Biofield, Tokyo, Japan) was subjected to HPLC analysis in a volume of 10 μl.

To quantitate total indoxyl sulfate, serum samples and dialysate samples were filtered through a 0.45-μm Chromatodisc 13 A filter, and 10 μl of the sample processed with the filter were subjected to high-performance liquid chromatography (HPLC).

To analyze the compounds that were not bound to serum protein, we passed serum and dialysate through a CF-25 ultrafiltration membrane filter (Amicon, Lexington, Mass., USA), then chromatographed a 10-μl sample of the ultrafiltrate. The ultrafiltration with the Amicon filter was facilitated by centrifuging at 1,000 *g* for 30 min.

High-Performance Liquid Chromatography

The chromatograph assembly from Japan Spectroscopic Co., Tokyo, Japan, consisted of a Model BIP-I pump equipped with a Model VL-614 injector, a variable wavelength detector, 875-UV, and a recorder, Chromatocorder 12.

To quantitate 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, a 25 cm × 4.6 mm Nucleosil 5-C18 reversed-phase column equipped with a 5 cm × 4.6 mm Nucleosil 5-C18 guard column (both from Chemco, Osaka, Japan) was used. A mobile phase acetonitrile/water/trifluoroacetic acid (40/60/0.08, by vol) was delivered at a flow rate of 1.0 ml/min at ambient temperature. The eluate was monitored by detection of absorbance at 270 nm.

To quantitate indoxyl sulfate, a 15 cm × 4.6 mm Pinkerton internal-surface reversed-phase (ISRP) column (particle size 5 μm) equipped with a 1 cm × 3 mm ISRP guard cartridge (both from Regis Chemical Co., Morton Grove, Ill., USA) was used. The mobile phase, 0.1 *M* KH_2PO_4/tetrahydrofuran (95/5, by vol), adjusted to pH 6.5 with 1 *M* NaOH, was delivered at a flow rate of 1.0 ml/min at ambient temperature. The eluate was monitored by detection of absorbance at 270 nm.

Erythroid Colony Assay

Marrow cells from C-57 Bl/6 mouse 8–10 weeks old were cultured at a concentration of 5×10^6 cells/ml in a culture medium containing α-modified Eagle's medium, 30% fetal calf serum, 0.825% methylcellulose, and 2.0 U/ml of erythropoietin. Cultures were incubated for 48 h at 37 °C in a humidified atmosphere at 95% air and 5% CO_2. After staining of the plates with diaminobenzidine, colony-forming unit-erythroid (CFU-E) of eight or more cells were counted using an inverted microscope. To determine the effects of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid and indoxyl sulfate on erythropoiesis, these were added into the culture medium at final concentrations of 200 μg/ml and 1 mg/ml, respectively. Quinolinic acid was added as a positive control into the culture medium at a final concentration of 200 μg/ml.

Results

Figure 1 shows the HPLC chromatograms of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid in uremic serum and uremic serum ultrafiltrate from CAPD patients, in normal serum, and in equilibrated peritoneal dialysate and dialysate ultrafiltrate from CAPD patients. Serum concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid in uremic patients on CAPD was increased as compared with that in normal subjects. The furancarboxylic acid in uremic serum was almost all bound to serum protein (albumin), since the compound could not be detected in uremic serum ultrafiltrate (fig. 1C). The furancarboxylic acid was detected in the equilibrated peritoneal dialysate, and its protein binding was about 60% (fig. 1E, F).

Figure 2 shows the HPLC chromatograms of indoxyl sulfate in uremic serum and serum ultrafiltrate from CAPD patients, in normal serum, and in equilibrated peritoneal dialysate and dialysate ultrafiltrate from CAPD patients. Serum concentration of indoxyl sulfate, the protein (albumin) binding of which was about 90%, was increased markedly in CAPD patients as compared with that in normal subjects (fig. 1B–D). Indoxyl sulfate was detected in the equilibrated peritoneal dialysate, and its protein binding was less than 10% (fig. 1E, F).

Serum concentrations of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, indoxyl sulfate, and creatinine, hematocrit and duration on dialysis were compared between uremic patients on CAPD and those on hemodialysis as shown in table 1. Serum level of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid in patients on CAPD was significantly lower than in those on hemodialysis, and the furancarboxylic acid was not removed by hemodialysis, since its serum level in patients on hemodialysis was increased after hemodialysis, due to concentration of serum protein by removing plasma water during hemodialysis. Serum concentration of indoxyl sulfate in CAPD patients was not different from that in hemodialysis patients. Hematocrit was significantly higher in CAPD patients than those in hemodialysis patients.

Table 2 shows removal of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, indoxyl sulfate and albumin by CAPD. Removal of 3-carboxy-4-methyl-5-propyl-

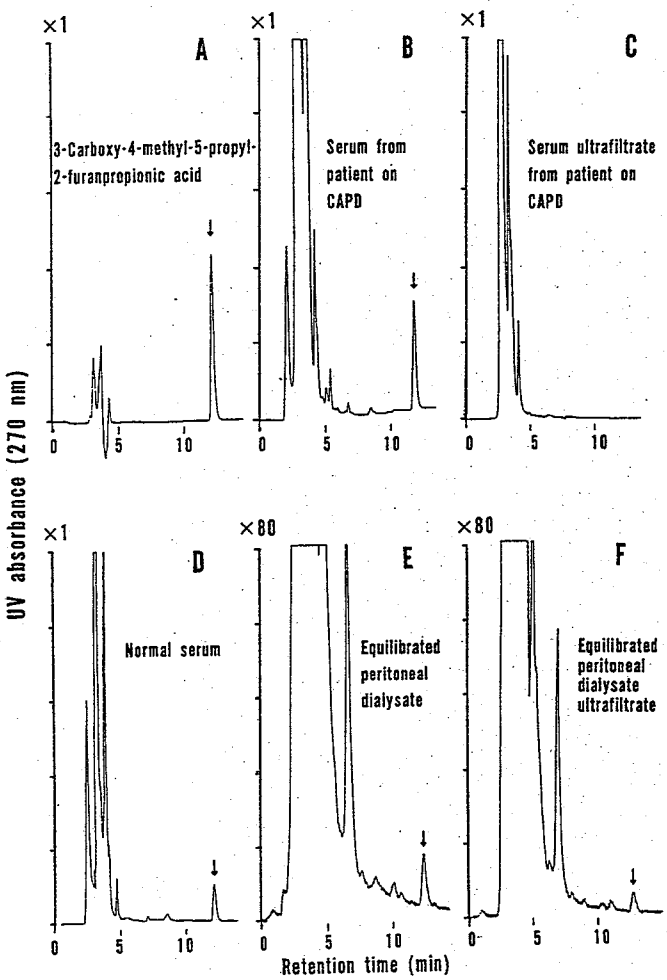

Fig. 1. High-performance liquid chromatograms of authentic 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (A), supernate of heat-deproteinized uremic serum from CAPD patients (B), uremic serum ultrafiltrate from CAPD patients (C), normal serum (D), equilibrated peritoneal dialysate (E) and equilibrated peritoneal dialysate ultrafiltrate (F).

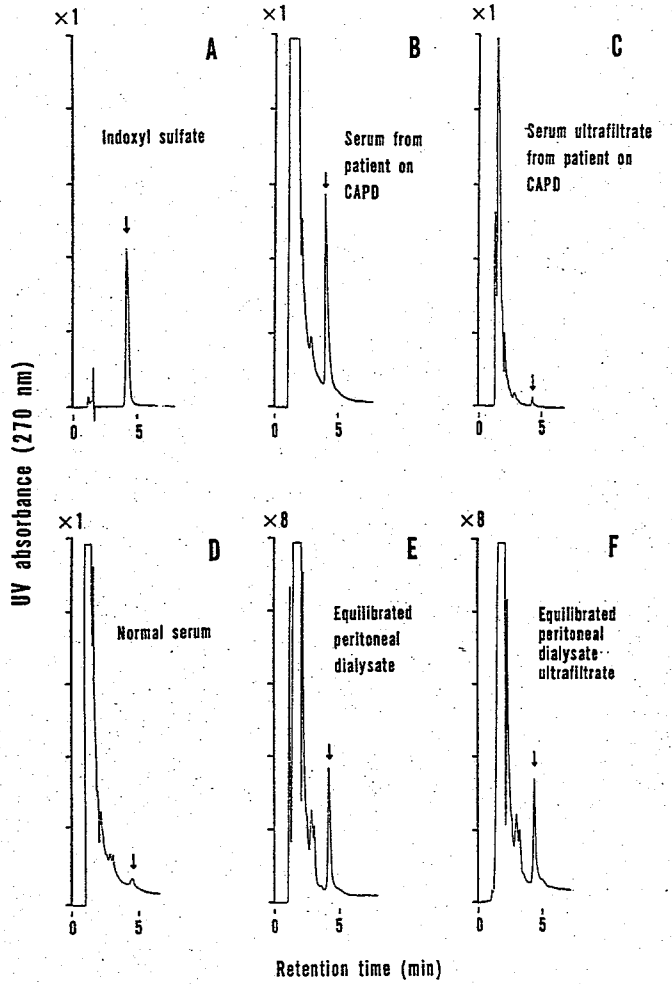

Fig. 2. High-performance liquid chromatograms of authentic indoxyl sulfate potassium salt (A), uremic serum from CAPD patients (B), uremic serum ultrafiltrate from CAPD patients (C), normal serum (D), equilibrated peritoneal dialysate (E) and equilibrated peritoneal dialysate ultrafiltrate (F).

Table 1. Serum concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid and indoxyl sulfate in CAPD and hemodialysis (HD) patients

	Normal (n=11)	CAPD (n=20)	HD (n=23)		p between CAPD and pre-HD
			pre-HD	post-HD	
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid, μg/ml	3.6±2.4	13.1±6.9	41.0±18.3	48.4±21.3	<0.001
Protein binding of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, %	98–100	98–100	98–100	98–100	
Indoxyl sulfate, μg/ml	0.5±0.27	20.3±12.2	27.6±16.5	22.7±12.5	NS
Protein binding of indoxyl sulfate, %	≃100	89.9±6.5	88.9±9.2	83.3±11.5	NS
Creatinine, μg/ml	<12	108±37	116±21	50±13	NS
Hematocrit, %	35~52	25.2±5.0	22.3±3.9		<0.05
Duration on dialysis, years		1.08±0.87	1.06±0.54		NS

Values represent mean±SD. NS=Not significant.

Table 2. Removal of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, indoxyl sulfate and albumin by CAPD (mean ± SD)

	Removal by CAPD mg/day	Protein binding %
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid	1.13 ± 0.42	55.2 ± 9.3
Indoxyl sulfate	30.2 ± 39.2	7.4 ± 7.9
Albumin	2,820 ± 580	

There were 8 subjects in each group.

Table 3. Urinary excretion of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid and indoxyl sulfate in healthy subjects (mean ± SD)

	Excretion mg/day
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid	1.24 ± 0.48
Indoxyl sulfate	61.8 ± 22.8

There were 20 subjects.

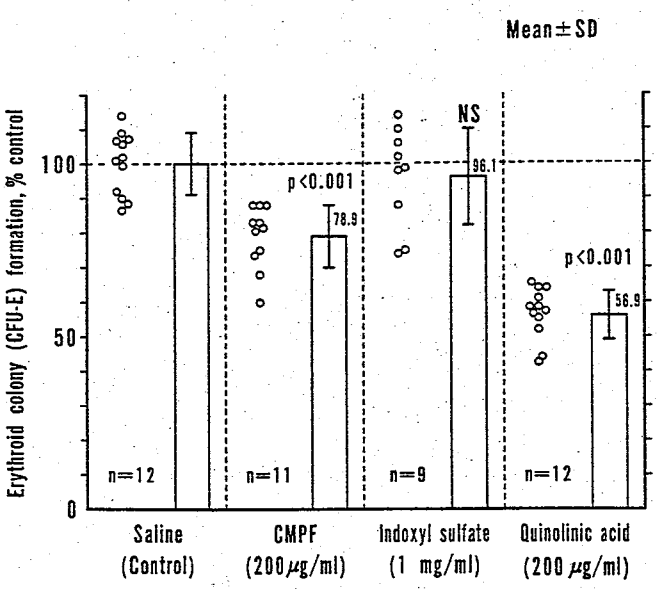

Fig. 3. The effects of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) and indoxyl sulfate on CFU-E. Quinolinic acid was included in the assay as a positive control.

2-furanpropionic acid as its albumin-bound form (about 60%) by CAPD was comparatively efficient, since it was close to the urinary excretory amounts in healthy subjects (table 3). Removal of indoxyl sulfate by CAPD was about half of the urinary excretion in healthy subjects (table 3).

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid showed a significant inhibition of erythroid progenitor cell CFU-E at a concentration of 200 μg/ml (fig. 3). Its maximum serum concentration observed in our uremic patients was 106.7 μg/ml. Indoxyl sulfate showed no inhibitory effect on CFU-E formation even at a concentration of 1 mg/ml. Quinolinic acid was simultaneously assayed for inhibition on CFU-E formation as a positive control [11].

Discussion

Serum concentration of 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid in CAPD patients was significantly lower than that in hemodialysis patients, whereas serum concentration of indoxyl sulfate in CAPD patients was not significantly different from that in hemodialysis patients. Although the reduction rate of indoxyl sulfate by hemodialysis was low as compared with that of creatinine, indoxyl sulfate could be removed by hemodialysis to a certain degree. However, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid could be hardly removed by hemodialysis due to its strong albumin binding. On the other hand, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid could be removed by CAPD with loss of albumin into dialysate.

In the present study, hematocrits were significantly higher in CAPD patients compared with hemodialysis patients. Factors that contribute to higher hematocrit in CAPD versus hemodialysis patients include a decrease of plasma volume [3, 12, 13], improved erythropoiesis [3, 9, 12–15], lesser iatrogenic blood loss, and improved red cell survival [12]. In most studies, the rise in hematocrit in CAPD patients has been related to improved erythropoiesis, which could result from increased erythropoietin production [14, 15], more efficient removal of inhibitors of erythropoiesis [9], and improved protein metabolism [16]. McGonigle et al. [6] suggested that the uremic toxins responsible for inhibition of erythropoiesis might be of small molecular size but not removed by dialysis due to intracellular or serum protein binding. As proposed by Wideröe et al. [9], the inhibitor of erythropoiesis might be a high-molecular-weight uremic toxin that could pass the peritoneal barrier.

We first demonstrated in this study that 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid inhibited erythroid progenitor cell CFU-E. The furancarboxylic acid has also been reported to show inhibition of albumin binding of drugs [2, 10, 17] and inhibition of hepatic

glutathione S-transferase [18]. The strongly albumin-bound furancarboxylic acid accumulated in uremic serum which could not be removed by conventional hemodialysis was found to be removed efficiently by CAPD as its albumin-bound form into peritoneal dialysate, resulting in its lower serum concentration in CAPD patients as compared with hemodialysis patients. These findings suggest that an efficient removal by CAPD of albumin-bound 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, an inhibitor of erythropoiesis, contributes to an improvement of hematocrit in CAPD patients.

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