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Short communication

Metabolic responses during hemodialysis determined by quantitative ^{1}H NMR spectroscopy

Masako Fujiwara[a,*], Itiro Ando[b], Kazuhisa Takeuchi[a,c], Shiro Oguma[d], Hiroshi Sato[b], Hiroshi Sekino[c], Keisuke Sato[d], Yutaka Imai[a]

[a] Department of Planning for Drug Development and Clinical Evaluation, Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aramaki-aza-aoba, Aobaku, Sendai 980-8578, Japan
[b] Laboratory of Clinical Pharmacology and Therapeutics, Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3, Aramaki-aza-aoba, Aobaku, Sendai 980-8578, Japan
[c] Koujinkai Central Clinic, 2-1-6, Tsutsujigaoka, Miyaginoku, Sendai 983-0852, Japan
[d] Koujinkai Nagamachi Clinic, 2-8-2, Happonmatsu, Taihakuku, Sendai 982-0001, Japan

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ABSTRACT

A large proportion of patients with end-stage renal disease have lifelong hemodialysis (HD) treatment. HD rapidly and indiscriminately removes necessary small metabolites together with uremic toxins from plasma into dialysate. To investigate metabolic responses to HD, we determined the levels of metabolites through time-course monitoring of ^{1}H NMR spectroscopy of dialysate during HD. The dialysate sample is stable for analysis because it contains only small metabolites without proteins. It was collected non-invasively from 9 HD patients with chronic glomerular nephropathy, at 6 time points during 4 h of HD in 5 sessions. Creatinine, alanine, lactate, pyruvate and valine were simultaneously quantified on a one-dimensional single-pulse spectrum with a single standard compound. The concentration of creatinine exhibited monotonous decay with time, while that of valine decreased slowly and then maintained its levels throughout an HD. Lactate, alanine and pyruvate increased at 2–3 h after the initiation of HD. They exhibited remarkable responses to HD with production from the body. The time-course of change in the 4 metabolites of lactate, pyruvate, alanine, and valine had reproducible behavior unique to each patient during the HD. This finding may be applied to distinguish metabolic status in HD patients.

1. Introduction

Most patients with end-stage renal disease have lifelong hemodialysis (HD) therapy in Japan.

In HD, extracorporeal circulation is applied to remove accumulated uremic toxins, electrolyte and fluid in plasma into the dialysate through a membrane during a 4 h HD session 3 times a week. The rate of exclusion in 4 h is approximately 10 times faster than that of previous accumulation for 2 or 3 days. In the therapy, not only toxins but also all the small metabolites of nutrients and physiologically necessary bioactive molecules are filtered indiscriminately [1]. The rapid removal of various metabolites in repeated therapies may cause profound metabolic stress to patients. In our previous study on ^{1}H NMR-based metabolomics of plasma from HD patients, post-HD plasma levels of lactate increased compared with pre-HD plasma levels [2]. The increments of lactate suggested production from the body during the HD.

To date, it has never been monitored with time how metabolites respond in the intermediate time of HD. In the present study, we investigated the time-course behavior of important metabolites which play a key-role in metabolic pathways related to lactate by ^{1}H NMR of dialysate through an HD.

The ^{1}H NMR technique for biofluid needs minimum sample preparation [2,3] unlike GC/MS or LC/MS methods. On a spectrum all the protons of various kinds of metabolites are detected with the same sensitivity. Spent hemodialysate consists of only small metabolites filtered proteins in plasma. We demonstrated in our previous study [3] that ^{1}H NMR of dialysate ensured quantification of the metabolites with enough sensitivity. A dailysate can be preserved stably at −25 °C for a long time because it is free from bacteria and enzymes. In the case of HD patients, blood collection is limited because they abolish kidney functions including hematogenesis and are mostly anemic. Dialysate collection was done non-invasively and frequently. Recently, we have verified dialysate to be an excellent surrogate for plasma in quantifying

* Corresponding author. Tel.: +81 22 795 4528; fax: +81 22 795 4532.
E-mail address: fmasako@m.tohoku.ac.jp (M. Fujiwara).

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small metabolites during HD by NMR analysis [3]. The quantitative ^{1}H NMR spectroscopy of dialysate offers a novel and simple tool for clinical investigations on HD patients.

2. Materials and methods

2.1. Patients

The study protocol was approved by the Ethical Committee of Koujinkai Hospital (Koujinkai Hemodialysis Clinic, Miyagi, Japan). Nine patients with maintenance HD were recruited and written informed consent was obtained from them. They were selected randomly from chronic glomerular nephropathy (CGN) patients undergoing stable HD without disorders of energy metabolisms such as diabetes mellitus. All patients were virtually anuric and dialyzed with high-performance polysulfone membranes. The flow rates of blood and dialysate were 200, and 500 mL/min, respectively. The total dialysate used in 4 h HD was 120 L.

2.2. Sample collection and preparation

From 2010 to 2012, dialysate were collected from the 9 patients during HD every 2 months for 10 months for each patient. Dialysate was collected at 15, 30 min, 1, 2, 3 and 4 (final) h after the initiation of HD in every session. Dialysates were sampled in standard plastic tubes and stored at −25 °C. They were thawed on ice immediately before use. For NMR measurement, 50 μl of deuterium oxide to provide a field-frequency lock and sodium 3-(trimethylsilyl) propionate 2, 2, 3, 3-d_4 (TSP) as an internal chemical shift and an intensity reference were added to the sample of 550 μl. Ten minutes after centrifugation of the sample at 13,000 rpm, we placed the supernatant into a 5 mm NMR tube.

2.3. Quantitative NMR spectroscopy

Single-pulse ^{1}H NMR spectra were recorded at 25 °C internal probe temperature using a 600 MHz NMR spectrometer (ECA600, JEOL Ltd., Tokyo, Japan). The water signal was suppressed by a presaturation pulse sequence, and the flip angle was set to 90°. Sixty-four scans were collected for 64 K data points with a spectrum-width 9 kHz. The repetition times were set to 42.4 s for dialysate, which ensured larger than $5 \times T_1$ [3]. Free induction decays were processed using the software 'Aline2 for Windows' (V. 6.0, JEOL Ltd.).

2.4. Quantification of metabolites

The signals of creatinine (CH_3, 3.05 ppm), lactate (CH_3, 1.33 ppm), pyruvate (CH_3, 2.38 ppm), alanine (CH_3, 1.47 ppm) and valine ($2CH_3$, 0.98, 1.04 ppm) for dialysate were assigned on the spectra referenced to TSP as a chemical shift standard (0.00 ppm), and quantified by integrations of these signal-areas referenced to TSP ($3CH_3$) as a quantitation standard as well. Glucose, acetate, and citrate were not analyzed here because they are composed in the original dialysate.

To obtain the plasma levels, the concentrations in dialysate were converted with the ratios derived in our previous study [3]. The levels can be roughly estimated by multiplication by a factor of 3.5, in common with metabolites.

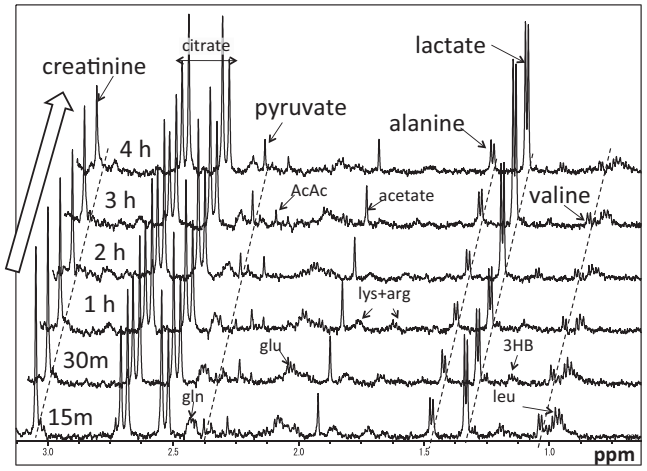

Fig. 1. Partial ^{1}H NMR spectra of dialysate during HD. Time-series of dialysate spectra measured by 1D single-pulse NMR in a typical patient were aligned where TSP peak heights were determined. The dialysate buffer used was citrate, thus citrate peaks were revealed to be constant during the 4-h session. (Conversely, when an acetate-containing buffer was used, acetate peaks were constant in the spectra.) AcAc: acetoacetate, 3HB: 3-hydroxybutyrate, gln: glutamine, glu: glutamate, lys: lysine, arg: arginine, leu: leucine.

Table 1
Baseline characters of study patients ($n = 9$).

Patient no.	1	2	3	4	5	6	7	8	9	Mean ± SD
Sex	F	F	F	M	M	F	M	F	F	
Age (yr)	70	61	58	73	55	69	64	60	75	65 ± 7
Weight (kg)	46.1 ± 0.6	52.6 ± 6.9	38.1 ± 0.1	44.3 ± 0.2	58.4 ± 1.1	51.7 ± 0.3	69.7 ± 1.1	55.3 ± 0.2	36.4 ± 0.7	50.3 ± 6.6
HD dutration (yr)	31	19	31	10	21	33	15	31	3	22 ± 11
dialysate	A/C	C	C	C	C/A	C	C	A	A	
Kt/V	1.59 ± 0.0	1.72 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.2	1.56 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.2
Creatinine (mg/dL)	9.8 ± 0.2	10 ± 0.9	9.7 ± 0.4	11.7 ± 0.2	12.7 ± 0.5	9.8 ± 0.6	17.2 ± 0.2	10 ± 0.4	8.5 ± 0.9	11.0 ± 2.5
Glucose (mg/dL)	80.8 ± 1.1	102 ± 8.8	99.8 ± 15	134 ± 39.7	96.6 ± 8.9	108 ± 11.8	132± 18.6	92.6 ± 8.4	86.8 ± 9.4	103.6 ± 17
Hemoglobin (g/dL)	11.3 ± 0.5	11 ± 0.7	9.6 ± 0.9	11.1 ± 0.3	11 ± 0.3	10.2 ± 0.6	11.1± 0.5	11.5 ± 0.3	11.2 ± 0.6	10.9 ± 0.6
Hematocrit (%)	33.8 ± 1.6	32.7 ± 1.9	28.5 ± 3.0	32.5 ± 1.1	32.4 ± 0.9	30.6 ± 2.0	33.6± 1.2	33.4 ± 1.0	32.9 ± 1.8	32.3 ± 1.6
Albumin (g/dL)	3.6 ± 0.2	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.16	4.0 ± 0.1	3.7 ± 0.2	4.01± 0.1	3.9 ± 0.1	3.5 ± 0.1	3.8 ± 0.2
Basal disease			TP				NS	IgA	SLE	

Clinical data are expressed as an average of 5 time measurements in each patient and values are mean ± S.D. In the right hand row, mean and S.D. of values in 9 patients are demonstrated.
In HD, heparin was used as the blood anticoagulant. During the HD session, supplemental nutrient was not added.
The dialysate buffer contained glucose (150 mg/dL), sodium (140 mEq), potassium (2 mEq), calcium (2.5–3 mEq), and citrate or acetate. A; bicarbonate dialysis buffers (8 mM acetate), C: dialysis buffer (0.7 mM citrate), A/C: 3 times of A and 2 times of C, C/A: 4 times of A and once C.
Kt/V: dialysis adequacy values estimated with the single-pool model described by Daugirdas [1].
Nine patients are CGN, which is a kind of syndrome. Some of them had basal diseases which are shown in the bottom line in the table. TP: toxemia of pregnancy, NS: nephrosclerosis, IgA: IgA nephropathy, SLE: systemic lupus erythematosus.