Nephrol Dial Transplant (2008) 23: 2304–2310
doi: 10.1093/ndt/gfn002
Advance Access publication 5 April 2008

Original Article

NDT
Nephrology Dialysis Transplantation

Influences of haemodialysis on the binding sites of human serum albumin: possibility of an efficacious administration plan using binding inhibition

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Abstract

Background. We have studied the possibility that low-dose treatment utilizing the inhibition that may occur between two drugs at the same site of human serum albumin (HSA) improves the pharmacological effects. The purpose is to elucidate the differences in the binding capacities of sites I and II of HSA between pre-haemodialysis (HD) and post-HD in patients with end-stage renal disease.

Methods. We evaluated free fractions of site probes, ^{14}C-warfarin (site I) and ^{14}C-diazepam (site II), by ultrafiltration in serum between pre-HD and post-HD. To investigate effects on the binding capacities of HSA sites, free fractions of site probes were calculated from the radioactivities measured with a liquid scintillation counter. Endogenous uraemic toxins, 3-carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF), indoxyl sulphate (IS) and hippurate (HA), were determined by HPLC. Free fatty acid (FFA) as an endogenous substance was determined with an automatic multi-item simultaneous analyser.

Results. The concentrations of HSA and FFA increased significantly (post-HD/pre-HD ratio: 1.18 ± 0.10, 5.46 ± 4.91), the concentrations of IS and HA decreased significantly (post-HD/pre-HD ratio: 0.69 ± 0.10, 0.33 ± 0.15) and CMPF concentrations did not alter significantly (post-HD/pre-HD ratio: 0.97 ± 0.12, $P = 0.471$). The free fractions of ^{14}C-warfarin decreased in all 14 patients at site I at post-HD compared to pre-HD (post-HD/pre-HD ratio: 0.59 ± 0.13). The free fractions of ^{14}C-diazepam at site II remarkably decreased in 10 of 14 patients (post-HD/pre-HD ratio: 0.61 ± 0.17) and unexpectedly increased in 4 (post-HD/pre-HD ratio: 1.08 ± 0.06) post-HD compared to pre-HD. In these four patients, when we investigated the influences of these variation factors on the reduction of the binding capacities of site II, [FFA]/[HSA] increased significantly post-HD, compared to pre-HD (post-HD/pre-HD ratio: 6.91 ± 6.58). ([FFA]/[HSA] ratios of the 4 patients were from 1.22 to 3.55, the highest for the 14 patients post-HD, but the ratios of the other 10 were below 1.2 post-HD.)

Conclusion. The binding capacity of site II was unexpectedly decremented by the effects of the remarkable elevation of FFA. Therefore, monitoring the binding capacity of site II in HD is important for patients with end-stage renal disease in the efficacious administration plan using the binding inhibition of HSA.

Keywords: binding site; free fatty acid; haemodialysis; human serum albumin; protein binding

Introduction

After absorption, drugs bind to serum proteins, although some differences exist in the degree of binding strength. Most high-affinity drugs bind to human serum albumin (HSA) [1] which accounts for 60% of serum proteins [2]. HSA has two major drug-binding sites, namely, sites I and II [3]. The drugs that bind to site I are warfarin and furosemide, while those that bind to site II are diazepam and diclofenac [4]. When two drugs bind to a common site, competitive inhibition may occur. As a result, the binding affinity of the inhibited drug decreases and the free concentration of the drug associated with the pharmacological effects increases. It was reported that the distribution of a drug to a target tissue is increased by the inhibitory effect of a drug binding to HSA [5]. An inhibitor that reduces the binding capacity of each site should possess the following properties: (1) a potent inhibitor of the protein binding of

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drugs; (2) when administered in large doses, its plasma concentration reaches high levels and (3) it is extremely safe and suitable for prolonged administration [6]. If we utilize the inhibition of the protein binding of drugs, the low-dose drugs could have a sufficient pharmacological effect.

We have demonstrated that bucolome improves the diuretic effect of furosemide. Bucolome ($K = 1.5 \times 10^6\ M^{-1}$), an inhibitor which reduces the binding capacity of site I, elevates the concentration of free furosemide ($K = 2.0 \times 10^5\ M^{-1}$) bound to the same site [6,7]. Figure 1 shows the distribution process of free furosemide by the inhibitory effect of bucolome. Furosemide binds to site I of HSA without bucolome in state 1. The binding of furosemide is inhibited by bucolome in state 2 (furosemide is distributed from vein to tissue). Consequently, an administration plan that utilizes the artificial decrease in the binding capacity of HSA sites with the coadministration of an inhibitor would have an efficacious pharmacological effect.

Since there has been a report that the binding affinity of a drug is changed by haemodialysis (HD) [8,9], we propose that it is important, rather than utilizing the artificial alteration of the binding affinity caused by an inhibitor, to instead utilize the spontaneous alteration of the binding affinity produced by HD. The present study was therefore undertaken to investigate the difference in the binding capacities of site I and site II of HSA between pre-HD and post-HD in patients with end-stage renal disease (ESRD).

Materials and methods

Samples (subjects)

The protocol was approved in advance by the Ethics Committee of Fujimoto Hayasuzu Hospital. The subjects were 12 males and 2 females, 20–74 (54.9 ± 16.0) years old, on HD for 4–116 (33.3 ± 8.3) months (Table 1). The cause of ESRD was diabetic nephropathy in eight patients, IgA nephropathy in one patient, Wegener's granulomatosis in one patient, purpuric nephropathy in one patient and unknown aetiology in three patients. The serum was extracted before and after HD from 14 renal failure patients, who participated as outpatients on HD. We referred to the clinical laboratory test results for the concentration of HSA of each patient.

The conditions of the haemodialysis sessions were as follows. All patients who were diagnosed with chronic renal failure (i.e. ESRD) and had a permanent arterial-venous fistula on the forearm underwent the usual haemodialysis using a polysulfone membrane (TORAYSULFONE TS-M, Toray Medical, Japan) with a duration of 4–4.5 h. The dialyzer surface area was 2.0 m^2 and replacement fluid (LYMPAKTA3, Nipro, Japan) was delivered after the membrane was inserted into the venous limb of the circuit. Blood was pumped through the membrane at a rate between 2.0 and 4.0 mL/kg/min. The dialysate was delivered countercurrently to the blood flow using a volumetric pump. Ultradiafiltrate flow was set at a rate of between 500 and 1300 mL/h. Heparin was administered as an anticoagulant throughout the procedure, and aspirin, aspirin-dialuminate, ticlopidine or warfarin was used as an antiplatelet agent. The dosage of heparin, the body weight between pre- and post-HD and the ultrafiltration volume are shown in Table 1. The medications were mainly antihypertensives (beta-blockers, calcium channel blockers, furosemide, ACE-inhibitors, ARBs), phosphate binders, $CaCO_3$, potassium-binders, proton-pump-inhibitors, H_2-blockers, insulin, hypoglycaemics and vitamin D supplements.

Protein binding study

Materials. Warfarin and diazepam radiolabelled with ^{14}C, [α-^{14}C-benzyl] warfarin (56 mCi/mmol) and [2-^{14}C] diazepam (55 mCi/mmol) were purchased from Amersham Biosciences (UK).

We obtained 3-Carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF) as a pure substance from Cayman Chemical (Ann Arbor, Michigan, USA). Indoxyl sulphate (IS) and hippurate (HA) were purchased as pure substances from Nacalai Tesque (Kyoto, Japan). All other chemicals were of analytical grade.

Measurement of free fraction using ultra-filtration. To investigate the variations in the binding of sites I and II of HSA between pre-HD and post-HD, ^{14}C-warfarin or ^{14}C-diazepam was added to the serum. The free fractions of radiolabelled site probes, ^{14}C-warfarin and ^{14}C-diazepam, in the serum were calculated by the ultrafiltration method and the following processes: The solution of a radiolabelled site-probe (14 μL) and saline solution (14 μL) was added to 700 μL of serum. Aliquots (10 μL) of the solution were placed in vials. The radioactivity of each solution before filtration was measured. Ultrafiltration experiments were performed using Minicent-10 centrifugal filter devices (Tosoh, Japan). A 200-μL volume of the solution was placed in the upper compartment. It was ultra-filtered at 3000 rpm for 10 min at room temperature using the centrifuge. A 10-μL solution isolated from the filtrate was counted as the radioactivity of the solution after filtration. The radioactivity of both solutions before filtration ($[RA_S]$) and after filtration ($[RA_F]$) was measured with a liquid scintillation counter (LSC-5000, Aloka, Japan) after adding scintillation fluid (ACSII, Amersham, UK) to scintillation vials.

The free fraction of the radiolabelled site probe was calculated as the per cent ratio of the activities of the solutions before and after filtration using the following equation [10]:

$$\text{Free fraction (\%)} = [RA_F]/[RA_S] \times 100$$

Endogenous substances assay and evaluation

Uraemic toxins assay. After 0.6 mL of acetonitrile was added to 0.3 mL of serum, mixed and churned for 10 min at room temperature, the mixed solution was centrifuged to remove protein at 3500 rpm for 10 min at room temperature. Then, 0.45 mL of the supernatant was separated after processing to remove protein. The concentrations of uraemic toxins, CMPF, IS and HA [11] in the serum were determined by HPLC (a system consisting of an LC-6A UV detector, Shimadzu; RP-18 Column, Waters) at a flow rate of 1 mL/min, using acetonitrile/0.2 M buffer of acetic acid/acetic acid (80:120:1 v/v) for CMPF and acetonitrile/0.2 M buffer of acetic acid (10:90 v/v) for IS and

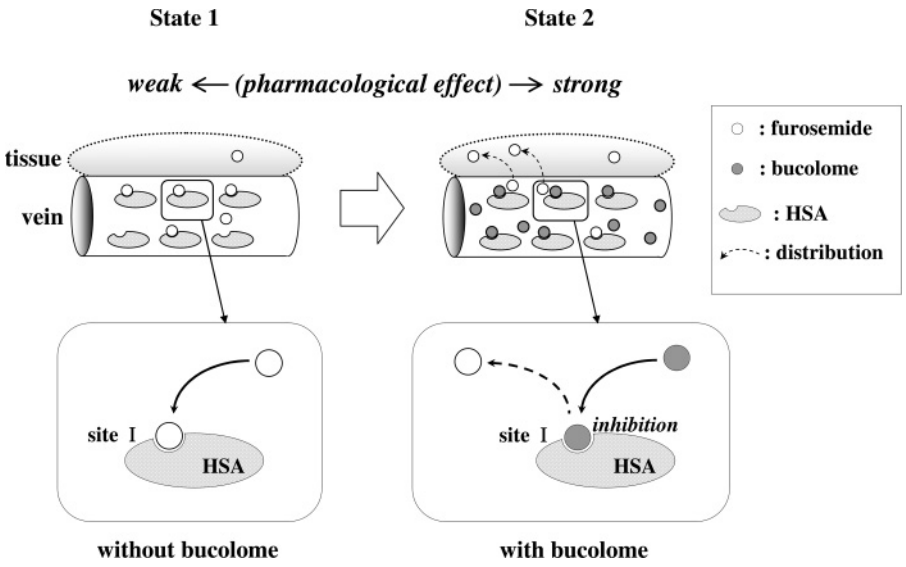

Fig. 1. Process of distribution of free furosemide by inhibitory effect of bucolome. State 1: binding of furosemide to serum albumin in the absence of bucolome. State 2: inhibition of furosemide by bucolome (increase of distribution to tissue).

Table 1. Patients on haemodialysis

Patient	Age	Sex (years)	Duration of HD (months)	Underlying disease[a]	Heparin dosage (Unit)	Body weight (kg)			Ultrafilteration volume (mL)
						Pre-HD	Post-HD	post/pre ratio	
1	70	M	24	Diabetes	5000	56.3	55.1	*0.98*	2100
2	57	F	116	IgA nephropathy	5000	46.2	43.6	*0.94*	3200
3	50	M	30	Diabetes	4000	61.7	59.8	*0.97*	2300
4	54	M	56	Wegener's granulomatosis	3000	54.6	51.2	*0.94*	4200
5	70	M	33	Diabetes	3000	50.3	47.1	*0.94*	3930
6	62	M	17	Purpuric nephropathy	4000	61.2	57.8	*0.94*	4100
7	28	M	28	Diabetes	4000	71.2	68.2	*0.96*	3400
8	74	F	20	Diabetes	4000	50.0	48.5	*0.97*	1600
9	65	M	40	Secondary hyperparathyroidism	4000	72.1	69.6	*0.97*	2800
10	39	M	18	Congenital myopathy	4000	46.3	43.8	*0.95*	2900
11	59	M	58	Diabetes	4000	61.8	58.6	*0.95*	3900
12	66	M	12	Diabetes	1500	53.0	51.0	*0.96*	3000
13	54	M	4	Hypertensive intracerebral haemorrhage	3000	66.3	63.6	*0.96*	3200
14	20	M	10	Diabetes	5000	54.8	52.5	*0.96*	2800
Mean	54.9		33.3		3821.4	0.48	1.13	*0.96*	3102.1
SD	16.0		8.3		952.9	0.77	0.93	*0.01*	773.04
Range	20–74		4–116		1500–5000	46.2–72.1	43.6–69.6	*0.94–0.98*	1600–4200

[a]All patients had end-stage renal disease.

HA as the mobile phase. A UV monitor was used to assay for CMPF, IS and HA at a UV wavelength of 261 nm, 285 nm and 240 nm, respectively.

Free fatty acid assay. The concentration of free fatty acid (FFA) was determined with an automatic multi-item simultaneous analyser, COBAS INTEGRA 400 plus (Roche Diagnostics, Switzerland).

Evaluation of endogenous substances. In general, the magnitude of the binding inhibition capacity of the HSA site is dependent on the ratio of the inhibitor concentration to a mole of HSA [12]. Accordingly, the procedure of evaluation by the ratio of [endogenous substance]/[HSA] was utilized since the level of inhibition of IS, HA and FFA was different between pre-HD and post-HD. The molecule weight of HSA was set as 66 500 [13,14].

Statistical analysis

Statistical significance was evaluated using Student's paired *t*-test to analyse differences between two groups, pre-HD

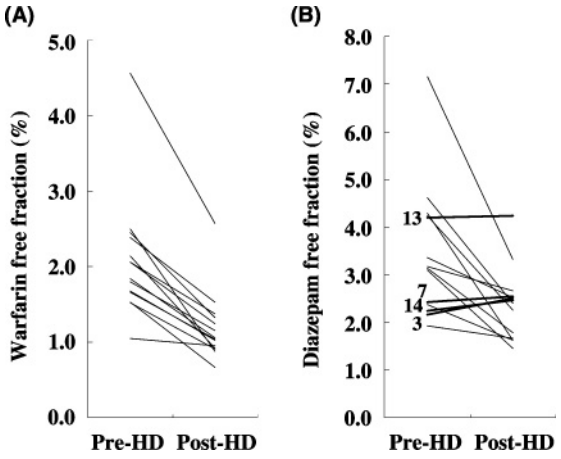

Fig. 2. Free fractions of ^{14}C-warfarin (**A**) and ^{14}C-diazepam (**B**) pre-HD and post-HD. (**A**) Lines are for expressing a variation in two values of free fractions of ^{14}C-warfarin pre-HD and post-HD. (**B**) Lines are for expressing a variation in two values of free fractions of ^{14}C-diazepam pre-HD and post-HD. Thick lines mean that the rate post-HD was higher than that pre-HD.

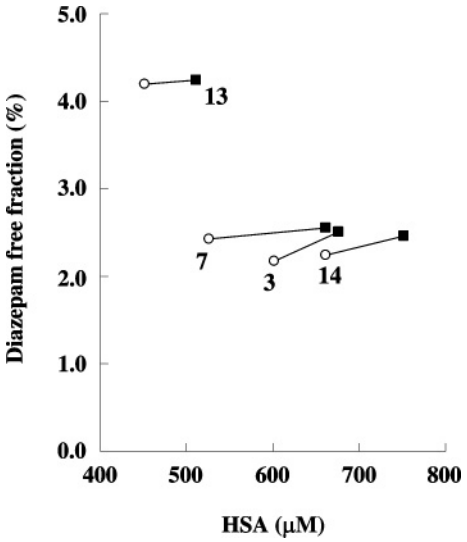

Fig. 3. The variation of ^{14}C-diazepam binding of HSA concentration between pre-HD and post-HD. Lines indicate each patient whose ^{14}C-diazepam free fraction increased post-HD. ◯: experimental values pre-HD, ■: experimental values post-HD.

and post-HD. The *P* value for statistical significance was set at <0.05. Data are expressed as mean ± S.D.

Results

Alterations in the binding capacities of sites I and II between pre-HD and post-HD

To evaluate the binding affinity of HSA sites I and II, respectively, the free fractions of ^{14}C-warfarin (site I) and ^{14}C-diazepam (site II) as site probes were calculated. Increases in the free fractions of ^{14}C-warfarin and ^{14}C-diazepam indicate decreases in the binding capacities of HSA sites I and II. The decrease in the free fraction indicates an increase in the binding capacity of the site. Figure 2A shows that the free fractions of ^{14}C-warfarin in all 14 patients decreased post-HD compared to pre-HD (post-HD/pre-HD ratio: 0.59 ± 0.13). The free fractions of ^{14}C-diazepam in 10 of 14 patients remarkably decreased post-HD compared to pre-HD (post-HD/pre-HD ratio: 0.61 ± 0.17), and the free fractions of ^{14}C-diazepam in 4 patients (nos. 3, 7, 13, 14) increased post-HD (post-HD/pre-HD ratio: 1.08 ± 0.06). In these four patients, HSA concentration post-HD was about 1.2 times as much as pre-HD (Figure 2B).

In general, drug binding affinity is increased at sites I and II of HSA because waste substances in the blood are removed by HD and the blood is purified [8,15]; however, the binding capacities of HSA site II in the four patients (nos. 3, 7, 13, 14) decreased post-HD. To elucidate the factors in the decreases of the binding capacities in these four patients, we investigated the effects of endogenous substances on HSA binding of ^{14}C-diazepam (^{14}C-diazepam free fraction).

Alterations in concentrations of HSA and endogenous substances

Table 2 shows the alterations in concentrations of HSA and endogenous substances. The concentration of HSA was significantly increased post-HD compared to pre-HD. This means that pre-HD serum was haemoconcentrated post-HD, i.e. indicating that the number of binding sites increased. In uraemic toxins (CMPF, IS and HA) and FFA, which have high binding affinities to HSA sites, the concentration of CMPF was not significantly altered but the concentrations of IS and HA were significantly reduced, and the concentration of FFA was significantly increased, post-HD compared to pre-HD. Therefore, we investigated

Table 2. Concentrations of HSA, CMPF, IS, HA and FFA ($n = 14$)

		Pre-HD patients (a)	Range	Post-HD patients (b)	Range	Ratio (b)/(a)	Range	Statistics (a) versus (b)
HSA	(μM)	554.2 ± 77.8	*436.1–706.8*	656.3 ± 105.9	*451.1–887.2*	1.18 ± 1.10	*1.03–1.45*	$P = 0.000006 < 0.00001$
CMPF	(μM)	78.4 ± 24.1	*27.5–112.5*	76.2 ± 25.6	*27.5–112.5*	0.97 ± 0.12	*0.67–1.09*	$P = 0.47$, N.S.
IS	(μM)	160.4 ± 69.0	*47.7–278.7*	108.8 ± 42.5	*33.0–188.8*	0.69 ± 0.10	*0.53–0.84*	$P = 0.00007 < 0.0001$
HA	(μM)	282.6 ± 341.0	*20.1–1395.5*	78.5 ± 89.1	*16.1–372.3*	0.33 ± 0.15	*0.16–0.80*	$P = 0.0099 < 0.01$
FFA	(μEq/L)	251.9 ± 359.3	*43.9–1344.0*	721.6 ± 528.6	*189.2–1939.7*	5.46 ± 4.91	*0.78–20.28*	$P = 0.002 < 0.005$

Each value represents the mean ± S.D. ($n = 14$). Statistical analysis was performed with paired Student's *t*-test. N.S.: not significantly different.

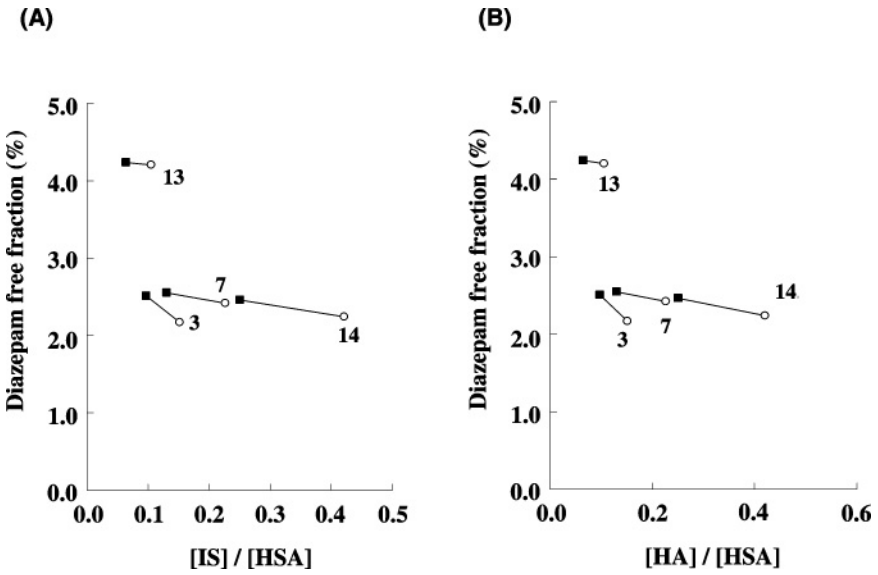

Fig. 4. Inhibition of uraemic toxins at site II of HSA; IS (**A**) and HA (**B**), ^{14}C-diazepam binding of HSA pre-HD and post-HD. (**A**) Lines indicate each patient whose ^{14}C-diazepam free fraction increased post-HD. ◯: experimental values (ratios of [IS]/[HSA]) pre-HD, ■: experimental values (ratios of [IS]/[HSA]) post-HD. (**B**) Lines indicate each patient whose ^{14}C-diazepam free fraction increased post-HD. ◯: experimental values (ratios of [HA]/[HSA]) pre-HD, ■: experimental values (ratios of [HA]/[HSA]) post-HD.

whether the effects on the binding capacity of HSA site II were due to HSA, IS, HA and FFA, which showed a significant difference in concentration.

Effects of concentrations of HSA and endogenous substances on binding capacity of site II

Effects of HSA concentrations. To evaluate the effects of HSA concentrations on the binding capacity of site II between pre-HD and post-HD, the relationship between the ^{14}C-diazepam free fraction and HSA concentration was investigated (Figure 3). The HSA concentrations increased about 100 μM post-HD as compared to pre-HD in the four patients. HSA concentrations post-HD were about 1.2 times those pre-HD, indicating an increase in the binding capacity of site II; however, the free fraction of ^{14}C-diazepam was increased in the four patients. It was suggested that the driving force of the decrement of the binding capacity of site II by other factors was greater than the driving force of the increment of the binding capacity of site II by the increase in HSA concentration. To evaluate the influences of IS, HA and FFA on the inhibitory effects on HSA site II, the relationship between the ^{14}C-diazepam free fraction and each of [IS]/[HSA], [HA]/[HSA] and [FFA]/[HSA] was investigated (see 'Evaluation of endogenous substances' under the 'Materials and methods' section).

Effects of uraemic toxin concentrations. To evaluate the effects of the uraemic toxins, IS and HA on the binding capacity of site II between pre-HD and post-HD, the relationship between the ^{14}C-diazepam free fraction and [IS]/[HSA] and [HA]/[HSA] was investigated. [IS]/[HSA] and [HA]/[HSA] decreased post-HD compared to pre-HD because IS and HA were removed from the blood by HD [16]. However, the ^{14}C-diazepam free fraction increased in spite of decreases in [IS]/[HSA] (post-HD/pre-HD ratio: 0.61 ± 0.03) and [HA]/[HSA] (post-HD/pre-HD ratio: 0.40 ± 0.21) in four patients (Figure 4A and B). It was suggested that the driving force of the decrement of the binding capacity of HSA site II by other factors was greater than that of the decrement of the binding capacity of site II by the decrease of IS and HA.

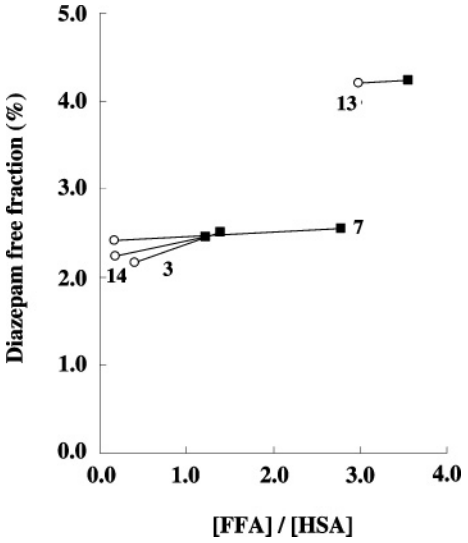

Fig. 5. Inhibition of FFA to ^{14}C-diazepam on site II of HSA pre-HD and post-HD. Lines indicate each patient whose ^{14}C-diazepam free fraction increased post-HD. ◯: experimental values (ratios of [FFA]/[HSA]) pre-HD, ■: experimental values (ratios of [FFA]/[HSA]) post-HD.

Effects of FFA concentrations on the binding capacity of site II. To evaluate the effects of FFA on the binding capacity of site II between pre-HD and post-HD,

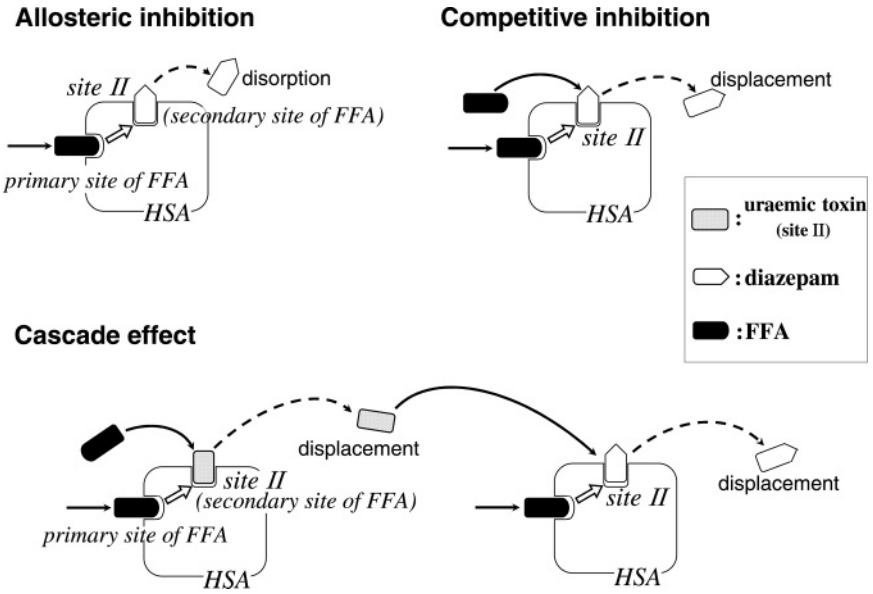

Fig. 6. In the four patients (nos. 3, 7, 13, 14), the mechanism of inhibitions pre-HD and post-HD. The allosteric and competitive inhibition: the allosteric inhibition mechanism of ^{14}C-diazepam bound to HSA site II by FFA. FFA binds to the primary site of FFA on HSA. FFA bound to the primary site of FFA on HSA provided an allosteric inhibition on ^{14}C-diazepam bound to site II (the secondary site of FFA). The competitive inhibition mechanism of FFA for ^{14}C-diazepam bound to HSA site II. FFA inhibits competitively at site II and displaces ^{14}C-diazepam from site II (direct process). Cascade effect: the cascade inhibition mechanism of FFA and uraemic toxins to ^{14}C-diazepam at HSA site II. Each FFA displaces uraemic toxins at HSA site II which is the secondary binding site of FFA allosterically and competitively. The displaced uraemic toxins competitively inhibit diazepam bound earlier to another HSA site II. Then, diazepam is displaced by competitive inhibition of the uraemic toxins. White arrows: allosteric inhibition at site II. Thin curved arrows: competitive inhibition at site II. Broken curved arrows: displacement or desorption from site II by inhibition.

the relationship between the ^{14}C-diazepam free fractions and [FFA]/[HSA] was investigated. The free fraction of ^{14}C-diazepam increased, and [FFA]/[HSA] also increased remarkably in the four patients (post-HD/pre-HD ratio: 6.91 $\pm$ 6.58). Each ratio of [FFA]/[HSA] post-HD was over 1.2 in the four patients (range of [FFA]/[HSA] ratio: 1.22–3.55) (Figure 5).

From the result above, it was suggested that the increase of the FFA concentration mainly contributed to the decrement of the binding capacities of site II in the four patients.

Discussion

When we estimated the binding capacities of sites I and II of HSA between pre-HD and post-HD, we found that, in all 14 patients post-HD, the binding capacities of site I increased and, unexpectedly, the binding capacities of site II did not increase. The binding capacities of site II in 4 of 14 patients decreased post-HD. It was suggested that the factors in the increase of the binding capacities of sites I and II were the increase in HSA concentration by haemoconcentration and the decrease in IS and HA concentration by HD. However, the cause of the decrease in the binding capacity of site II at post-HD was not the increment of the HSA concentration or the decrease in the IS and HA concentrations, but rather the remarkable increase in the FFA concentration in the four patients. The inhibitory effects of FFA were considered to exceed the promoting effect on the binding capacity of HSA site II. The inhibition mechanism of FFA for the unique reduction of the binding capacity of site II is discussed below.

Inhibition mechanisms of FFA for the reduction of the binding capacity of site II

In general, the heparin used in HD activates lipase in the blood and increases the concentration of FFA remarkably [17–19]. It has been reported that the binding capacity of site II was decreased by increasing FFA concentration [20]. From these reports, there is no doubt that the increment of FFA contributed to the reduction of site II binding capacity.

In the four patients (nos. 3, 7, 13, 14), it was assumed that FFA competitively inhibited ^{14}C-diazepam bound to site II of HSA, because the region of site II coincides with the secondary binding site of FFA on HSA and the binding constant K value of the secondary site for FFA, 10^7 M^{-1} [21] is greater than that of diazepam, 3.8×10^5 M^{-1} [22]. Therefore, when the ratio [FFA]/[HSA] increases, FFA, which retains high binding affinity to HSA, also inhibits site II (the secondary site of FFA), to which ^{14}C-diazepam bound directly [23]. Thus, the inhibitory mechanism post-HD was considered to add not only allosteric inhibition (weak) but competitive inhibition (strong) [8], since the ratio [FFA]/[HSA] in three of the four patients (nos. 3, 7, 14) was <0.5 pre-HD and >1.2 post-HD. The binding capacity of site II post-HD in comparison with that of pre-HD is remarkably influenced by the change of these allosteric and competitive inhibitions. Because the [FFA]/[HSA] ratio of one (no. 13) of the four patients pre-HD was high at 2.98, the binding inhibition of FFA at site II was considered to contribute not only to allosteric inhibition but also to the competitive inhibition. The effect of competitive inhibition in site II post-HD would be therefore greater than its

inhibition pre-HD, since the ratio [FFA]/[HSA] increased to 3.55 post-HD. In those cases, if uraemic toxins bound to site II when competitive inhibition of site II was produced by increasing FFA remarkably, the uraemic toxins would be inhibited by increasing FFA and would bind to the other HSA site II, possibly displacing ^{14}C-diazepam competitively. Thus, it was suggested to be a good possibility that the mechanism of the synergistic inhibitory 'cascade effect' was produced by the chain inhibition of FFA and uraemic toxins at site II [8].We considered that unexpected reduction of the binding affinity of site II in the four patients was produced by adding the allosteric inhibition to competitive inhibition of FFA and by the 'cascade effect' of FFA and uraemic toxins (see Figure 6).

Strategy of administration

In dosage plans that utilize binding inhibition, we consider that if a drug binding with high affinity to site I is administered pre-HD, the free fraction of the drug can be increased. On the other hand, since a drug binding to site II is sometimes inhibited by increasing FFA remarkably post-HD, we consider that a drug binding to site II of HSA should be medicated with the optimum timing. A drug that could utilize the drug binding inhibition of HSA for effective administration plans should possess the properties of potent protein binding, with a small volume of distribution and high distribution to the target tissue. It is expected that a drug with these properties can increment distribution to the target tissue by utilizing the binding inhibition on sites I and II of HSA by HD. One drug that can be used for efficacious administration using binding inhibition is ketoprofen, which is used as an analgetic and antiphlogistic for the pain and inflammation of arthrosteitis in HD patients, as an example. It has been reported that this drug binds to site II of HSA strongly and is greatly inhibited by increasing uraemic toxins and FFA in HD patients. Therefore, we consider that ketoprofen may be highly distributed to inflammatory tissue by using the inhibition of protein binding.

In conclusion, this study elucidated that a significant increase in FFA sometimes reduces the binding capacity of HSA site II post-HD compared to pre-HD. Consequently, we conclude that monitoring the binding capacity of site II of HSA between pre-HD and post-HD is clinically important for patients with ESRD in planning efficacious administration utilizing the binding inhibition of HSA.

Conflict of interest statement. None declared.

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Received for publication: 11.5.07
Accepted in revised form: 31.12.07