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Research Paper

Cross-linked human serum albumin dimer has the potential for use as a plasma-retaining agent for the fatty acid-conjugated antidiabetic drugs

Kazuaki Taguchi[a], Victor Tuan Giam Chuang[b], Keishi Yamasaki[a,g], Yukino Urata[c], Ryota Tanaka[c], Makoto Anraku[a], Hakaru Seo[a,g], Keiichi Kawai[d], Toru Maruyama[c,e], Teruyuki Komatsu[f] and Masaki Otagiri[a,c,g]

[a]Faculty of Pharmaceutical Sciences and [g]DDS Research Institute, Sojo University, [c]Department of Biopharmaceutics and [e]Center for Clinical Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, [d]School of Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, [f]Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, Bunkyo-ku, Japan and [b]School of Pharmacy, Faculty of Health Sciences, Curtin University, Perth, Western Australia, Australia

Keywords
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Correspondence
Masaki Otagiri, Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto 862-0082, Japan.
E-mail: otagirim@ph.sojo-u.ac.jp

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Abstract

Objectives The half-life of fatty acid-conjugated antidiabetic drugs are prolonged through binding to albumin, but this may not occur in diabetic patients with nephropathy complicated with hypoalbuminemia. We previously showed that human serum albumin (HSA) dimerized at the protein's Cys34 by 1,6-bis(maleimido)hexane has longer half-life than the monomer under high permeability conditions. The aim of this study was to investigate the superior ability of this HSA dimer as a plasma-retaining agent for fatty acid conjugated antidiabetic drugs.

Methods The diabetic nephropathy rat model was prepared by administering a single injection of streptozotocin (STZ) intravenously, and the pharmacokinetic properties of HSA monomer and dimer were evaluated. Site-specific fluorescent probe displacement experiments were performed using warfarin and dansylsarcosine as site I and site II specific fluorescent probes, respectively.

Key findings The half-life of the HSA dimer in STZ-induced diabetic nephropathy model rats was 1.5 times longer than the HSA monomer. The fluorescent probe displacement experiment results for HSA monomer and dimer were similar, where fatty acid-conjugated antidiabetic drugs displaced dansylsarcosine but not warfarin in a concentration-dependent manner.

Conclusions The HSA dimer shows potential for use as a plasma-retaining agent for antidiabetic drugs due to its favourable pharmacokinetic properties.

Introduction

There were approximately 285 million cases of diabetes which is equivalent to ~6.4% of the world population making it one of the most common diseases in the world,[1] and as the disease progresses, diabetic patients are commonly associated with complications such as diabetic polyneuropathy, retinopathy, nephropathy and angiopathy. New classes of medicines such as dipeptidyl peptidase-4 inhibitors, glucose transporter inhibitors and insulin preparations have been developed to better control the blood sugar level as well as to improve quality of life. Among these new drugs, insulin analogues (insulin detemir, insulin degludec) and glucagon-like peptide-1 (GLP-1) receptor agonist (liraglutide), have gained a place in diabetic treatment worldwide recently. These drugs reversibly bind albumin through the long-chain fatty acid attached to them (insulin or mutated GLP-1 with 97% homology to native GLP-1). As a result, they distribute more slowly from the injection site to peripheral target tissues thus extending their half-life[2–4] (insulin detemir; 5–7 h, insulin degludec; <24 h vs native insulin; 4–6 min, liraglutide; 13 h vs GLP-1; <2 min) and preventing them from degradation in circulation as well as avoiding glomerulus filtration.[3] These properties allow them for once or twice-daily dosing.

Clinically, more than 40% of diabetic patients have diabetic nephropathy. As the diabetic nephropathy progressed to end-stage renal failure, continuous protein leakage through glomerulus and high capillary protein permeability took place, accompanied by an increased albumin flux to the extravascular compartment. Consequently, patients with diabetic nephropathy will also be affected by hypoalbuminemia at the same time. Hence, the half-lives of fatty acid-conjugated insulin analogues and GLP-1 receptor agonist may be shorter in diabetic nephropathy patients complicated with hypoalbuminemia, which may result in inadequate blood sugar control. Although human serum albumin (HSA) infusion may be a therapeutic option for these patients, the infused albumin is expected to rapidly distribute to the extravascular compartment due to high capillary protein permeability and increased glomerulus filtration. Therefore, a new albumin preparation that can maintain albumin concentration in the circulation of patients with diabetic nephropathy and also preserves drug binding capacity is highly desirable.

In general, increasing the molecular size of albumin by polymerization and PEGylation and others is widely recognized as the best strategy for reducing glomerulus filtration and high capillary permeability.[5–7] Although these measures could prolong the blood retention of albumin polymers, the drug binding capacity HSA is compromised. We previously reported HSA dimer prepared by cross-linking two HSA molecules with 1,6-bis(maleimido)hexane (BMH)[8] showed superior plasma retention property compared with HSA monomer in the doxorubicin-induced nephrotic rat model.[9] In addition, the length of BMH seems to be sufficiently long (16.1 Å), permitting HSA dimer to preserve the structural properties of native HSA monomer including ligand binding capacity and blood compatibility.[8,10] These evidence had led us to hypothesize that HSA dimer produced from two HSA molecules chemically linked with BMH has the potential as a plasma-retaining agent of antidiabetic drugs, such as fatty acid conjugated insulin analogues and GLP-1 receptor agonist, for treating hypoalbunemia under diabetic nephropathy condition. A rat model of diabetic nephropathy induced by treatment with streptozotocin (STZ) was used to evaluate the pharmacokinetic properties of HSA monomer and HSA dimer. In addition, the binding ability of HSA dimer for fatty acid-conjugated insulin analogues (insulin detemir and insulin degludec) and GLP-1 receptor agonist (liraglutide) was determined.

Materials and Methods

Chemicals

HSA (Lot. U389PX, 25% wt.) was obtained from BENESIS (Osaka, Japan). Ethanol and dithiothreitol were purchased from Wako Pure Chemical Industries Ltd (Tokyo, Japan) and used without further purification. BMH was purchased from Thermo Fisher Scientific, Inc. (Rockford, IL, USA). $^{111}InCl_3$ (74 MBq/ml in 0.02 N HCl) was donated by Nihon Medi-Physics (Takarazuka, Japan). STZ was purchased from Nakarai Tesque (Lot. M3M2485, Kyoto, Japan). All other chemicals were of the highest grade commercially available, and all solutions were prepared using deionized distilled water. The HSA dimer was synthesized according to procedures we have reported previously.[8]

Preparation of streptozotocin-induced diabetic nephropathy model rat

All animal experiments were performed according to the guidelines, principles and procedures for the care and use of laboratory animals of Kumamoto University (Approval No.C20-206). Sixteen male Sprague–Dawley (SD) rats (5 weeks old, Kyudou Co., Kumamoto, Japan) were used in this study. All animals were maintained under conventional housing conditions, with food and water *ad libitum*, in a temperature-controlled room with a 12-h dark/light cycle.

A single dose of STZ (60 mg/kg) given intravenously to rats has been reported to produce clear diabetic symptoms,[11] hence 11 SD rats were administered STZ at a dose of 60 mg/kg through the tail vain under ether anaesthesia. Five SD rats were used as control group.

Assessment of diabetic nephropathy model rat induced by streptozotocin

One day before the experiments (13 days after STZ administration), urine was collected for 24 h in a metabolic cage, and the volume of urine was measured. After urine collection was completed (14 days after STZ administration), five STZ-induced diabetic nephropathy model rat and five control rats were anaesthetized with ether, and blood samples were collected from the inferior vena cava. The blood samples were centrifuged (3000 g, 10 min) to obtain plasma for the analysis of serum creatinine (Scr) and blood urea nitrogen (BUN) and glucose. Mean Scr, BUN and glucose concentrations were measured by LaboAssay Creatinine, UNB-test Wako and GlucoseCII-test Wako (Wako Pure Chemical Industries, Ltd, Osaka, Japan) assays, respectively. After blood samples were collected, the rats were sacrificed by acute bleeding from the abdominal aorta and to obtain the kidney and pancreas for histopathological studies. The organs were fixed in 4% paraformaldehyde overnight.

Histopathological examination

The fixed kidneys and pancreases were embedded in paraffin, sectioned into 5-μm slices and then stained with

Periodic acid-Schiff (PAS) and H&E, respectively on a slide. The slide was observed using Microscope (Keyence, BZ-8000, Osaka, Japan).

Proteins labeling with ^{111}In

For the pharmacokinetic experiments, the HSA monomer and dimer were radiolabeled with ^{111}In as previously reported.[9] Both ^{111}In-labeled HSA monomer and dimer were mixed with unlabeled HSA monomer and dimer, respectively, to adjust the protein concentration before use in the pharmacokinetic experiments.

The pharmacokinetic experimental protocol in streptozotocin-induced diabetes model rat

Fourteen days after STZ administration, the rats were used as a STZ-induced diabetic nephropathy model rat. All STZ-induced diabetic nephropathy model rat were anaesthetized using ether and received a single injection of ^{111}In-labeled HSA monomer in saline ($n = 3$, 1 mg/kg, 2 ml/kg, 1×10^7 cpm/kg) or HSA dimer in saline ($n = 3$, 1 mg/kg, 2 ml/kg, 1×10^7 cpm/kg) via the tail vein. At each time point (3 min, 10 min, 30 min and 1, 2, 6, 14, 18, 24, 30, 38, 42 and 48 h) after an injection of the ^{111}In-labeled protein, a 100 μl aliquot of blood was collected from the tail vein, and plasma was obtained by centrifugation (6000 *g*, 5 min). At 48 h after the injection of the ^{111}In-labeled protein, the rats were sacrificed, and the organs were collected and rinsed with saline. Urine was collected at fixed intervals in a metabolic cage. The levels of ^{111}In in the plasma, excised organs and urine were determined using a gamma counter (ARC-5000; Aloka, Tokyo, Japan).

Fluorescent probe displacement experiments

Warfarin potassium (WF) (Eisai Co., Tokyo, Japan) and dansylsarcosine (DNSS) (Sigma, St. Louis, MO, USA) were used as the fluorescent probes for site I and site II, respectively.[12] Both probes were dissolved in 100% ethanol. The displacement experiments of WF and DNSS by insulin analogues (insulin detemir; Levemir (novo nordisk, Bagsvaerd, Denmark), insulin degludec; Tresiba (novo nordisk, Bagsvaerd, Denmark)) and GLP-1 receptor agonist (liraglutide; Victoza (novo nordisk, Bagsvaerd, Denmark)) were performed by recording the fluorescence of a solution containing 2 μM WF (or DNSS) and 2 μM HSA monomer (or 1 μM HSA dimer) while gradually increasing the insulin analogues (or GLP-1 receptor agonist) concentration. The excitation wavelengths for WF and DNSS were 320 and 350 nm, respectively. The emission spectra for WF and DNSS were recorded in the range of 350–450 nm and 400–600 nm, respectively.

Data analysis

Pharmacokinetic analyses after the administration of ^{111}In-labeled HSA monomer or dimer were carried out using a two-compartment model. Pharmacokinetic parameters were calculated by curve fitting using a nonliner least squares program (MULTI) for microcomputers (http://www.pharm.kyoto-u.ac.jp/byoyaku/Kinetics/download.html).[13] Data are shown as the mean ± SD for the indicated number of animals. Significant differences among each group were determined using the two-tail unpaired Student's *t*-test. A probability value of $P < 0.05$ was considered to be statistically significant.

Results

Assessment of a rat model of diabetes induced by streptozotocin

To evaluate the extent of pancreas impairment of the STZ-treated rats, we measured the changes of body weight, the 24-h urine collection volume, the levels of glucose in plasma. As a result, the STZ-treated rats showed body weight loss (307.5 ± 9.6 and 251.3 ± 20.9 g, $P < 0.01$, for control and STZ-treated rat, respectively, Figure 1a) and increased volume of 24-h urine collection (34.5 ± 11.6 and 109.5 ± 36.0 ml/day, $P < 0.01$, for control and STZ-treated rat, respectively, Figure 1b) at 14 days after STZ treatment when compared with the control rats. The glucose levels in the STZ-treated rats were significantly increased by approximately 1.5-fold compared with that in the control rats (194.2. ± 2.3 and 301.6 ± 90.1 mg/dl, $P < 0.05$, for control and STZ-treated rat, respectively, Figure 1c). Furthermore, degenerative changes and clear cytoplasm in the pancreatic islet of the STZ-treated rats was observed from the H&E staining data, but not in the control rats (Figure 1d).

Scr and BUN levels were measured to determine the degree of renal impairment of the STZ-treated rats. The Scr and BUN levels were not significantly different between control and STZ-treated rats (Figure 1e and 1f). However, glomerulus damage was clearly observed in the STZ-treated rats from the PAS staining data, but not in the control rats (Figure 1g).

Pharmacokinetic experiment in streptozotocin-induced diabetes model rat

Pharmacokinetic properties of the HSA monomer and dimer were examined in the STZ-induced diabetic nephropathy model rats. The volume of 24-h urine collection in STZ-induced diabetic model rats used in the pharmacokinetic study was 122.0 ± 22.8 ml (monomer; 113.8 ± 24.6 ml, dimer; 130.2 ± 22.2 ml, not statistically significant difference).

Figure 2a shows the plasma concentration time course of the ^{111}In-HSA monomer and ^{111}In-HSA dimer administered

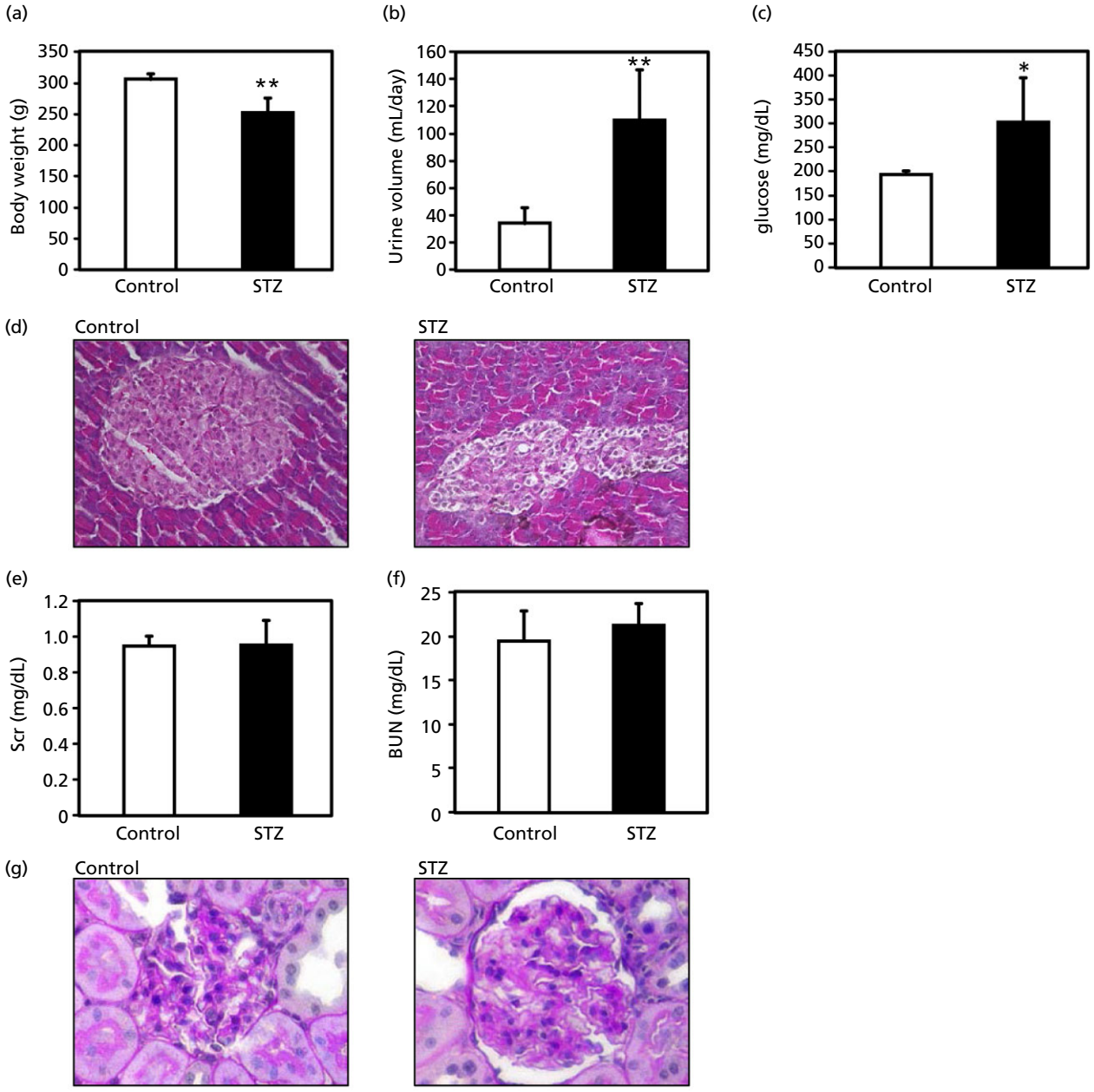

Figure 1 The parameters (a) body weight, (b) urine volume and (c) glucose in plasma in control rats (opened column) and streptozotocin-treated rats (closed column). (d) Light micrographs of pancreas stained with H&E. The parameters of (e) serum creatinine and (f) blood urea nitrogen in plasma in control rats (opened column) and streptozotocin-treated rats (closed column). (g) Light micrographs of kidney stained with Periodic acid-Schiff stain. The Sprague–Dawley rats were administered streptozotocin as a single injection (60 mg/kg) through the tail vain. Urine was starting to be collected for 24 h in a metabolic cage at 13 days after streptozotocin administration. Fourteen days after the streptozotocin injection, biochemical parameters and histological study were evaluated. The values are the mean ± SD. ($n = 5$) *$P < 0.05$ vs control rats, **$P < 0.01$ vs control rats.

to the STZ-induced diabetic nephropathy model rats at a dose of 1 mg/kg, and Table 1 lists the pharmacokinetic parameters obtained using a two-compartment model. The plasma concentration of HSA monomer was rapidly cleared compared with that of the HSA dimer in diabetic nephropathy rats (Figure 2a). The clearance for the HSA dimer decreased to half of the HSA monomer (0.61 ± 0.07 and 0.34 ± 0.06 ml/h, $P < 0.01$, for the HSA monomer and dimer, respectively). The $t_{1/2}$ and the area under the concentration-time curve (AUC) of the HSA dimer

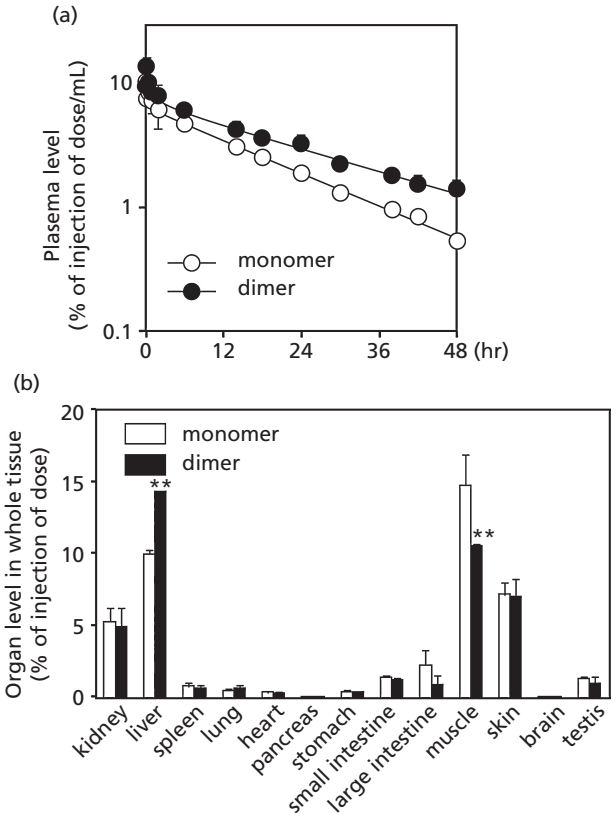

Figure 2 (a) Plasma concentration of ^{111}In-HSA monomer (open circles) and dimer (closed circles) after intravenous administration to streptozotocin-induced diabetic nephropathy model rats. (b) Tissue distribution of radioactivity in each whole tissue (% of injection of dose) at 48 h after intravenous administration of ^{111}In-HSA monomer (open bars) or dimer (closed bars) to streptozotocin-induced diabetic model rats. Fourteen days after the streptozotocin injection, all streptozotocin-induced diabetic nephropathy model rats received a single injection of ^{111}In-HSA monomer or dimer at a dose of 1 mg/kg. Each point represents the mean ± SD. ($n = 3$) $^{**}P < 0.01$ vs human serum albumin monomer.

Table 1 Pharmacokinetic parameters after administration of ^{111}In-human serum albumin monomer and dimer to diabetic nephropathy model rats

parameters	^{111}In-HSA monomer	^{111}In-HSA dimer
$t_{1/2}$ (h)	14.1 ± 1.2	21.1 ± 1.8**
AUC (h×% of dose/ml)	165 ± 19	298 ± 49**
ke (hr-1)	0.076 ± 0.005	0.050 ± 0.006**
CL (ml/h)	0.61 ± 0.07	0.34 ± 0.06**
Vd (ml)	11.6 ± 1.1	9.8 ± 1.8
MRT (h)	18.4 ± 1.3	29.2 ± 2.1**

AUC, area under the concentration-time curve; CL, clearance; HAS, human serum albumin; MRT, mean residence time; $t_{1/2}$, half-life; Vd, volume of distribution at steady state. Each value represents the mean ± SD. ($n = 3$) $^{**}P < 0.01$ vs ^{111}In-HSA monomer.

increased compared with the HSA monomer ($t_{1/2}$, 14.1 ± 1.2 and 21.1 ± 1.8 h, $P < 0.01$ and AUC, 165 ± 19 and 298 ± 49 h % of dose/ml, $P < 0.01$, for the HSA monomer and dimer, respectively) (Table 1).

The tissue distribution (kidney, liver, spleen, lung, heart, pancreas, stomach, small intestine, large intestine, muscle, skin, brain and testis) of the ^{111}In-HSA monomer and ^{111}In-HSA dimer at 48 h after administration was also examined. As shown in Figure 2b, both HSA monomer and dimer mainly distributed to the liver and muscle. The accumulation of HSA dimer in muscle was lower compared with the HSA monomer (14.8 ± 2.1 and 10.5 ± 0.1% of dose, $P < 0.01$, for the HSA monomer and dimer, respectively), but HSA dimer in the liver was significantly higher than HSA monomer (10.0 ± 0.3 and 14.3 ± 0.03% of dose, $P < 0.01$, for the HSA monomer and dimer, respectively). There were no significant differences between HSA monomer and dimer in other organs (Figure 2b). In addition, the radioactivity of ^{111}In after administration of the HSA monomer and dimer were approximately 8% and 5% of dose at 48 h, respectively.

Fluorescent probe displacement experiments

To function as a retaining agent for fatty acid conjugated insulin analogues and GLP-1 receptor agonist, HSA dimer must maintain similar ligand binding capacity as HSA monomer. Therefore, site-specific fluorescent probes displacement experiments in HSA monomer and dimer were performed using WF and DNSS as site I and site II-specific fluorescent probes, respectively. Addition of fatty acid-conjugated insulin analogues and GLP-1 receptor agonist to the HSA proteins–fluorescent probes mixtures caused a concentration-dependent reduction in the DNSS fluorescence intensity but did not cause a reduction of the WF fluorescence intensity in both HSA monomer and dimer (Figure 3).

Discussion

The results in this study showed that HSA dimer has a longer blood retention property compared with HSA monomer in the diabetic nephropathy rat model, possibly through suppression of the infused HSA dimer flux to the extravascular compartment (Figure 2). In addition, HSA dimer has identical ligand binding capacity for the fatty acid conjugated insulin analogues and GLP-1 receptor agonist as the HSA monomer (Figure 3). These findings suggested that HSA dimer produced by linking two HSA molecules with BMH chemically can function as a plasma-retaining agent of the fatty acid conjugated antidiabetic drugs under diabetic nephropathy condition.

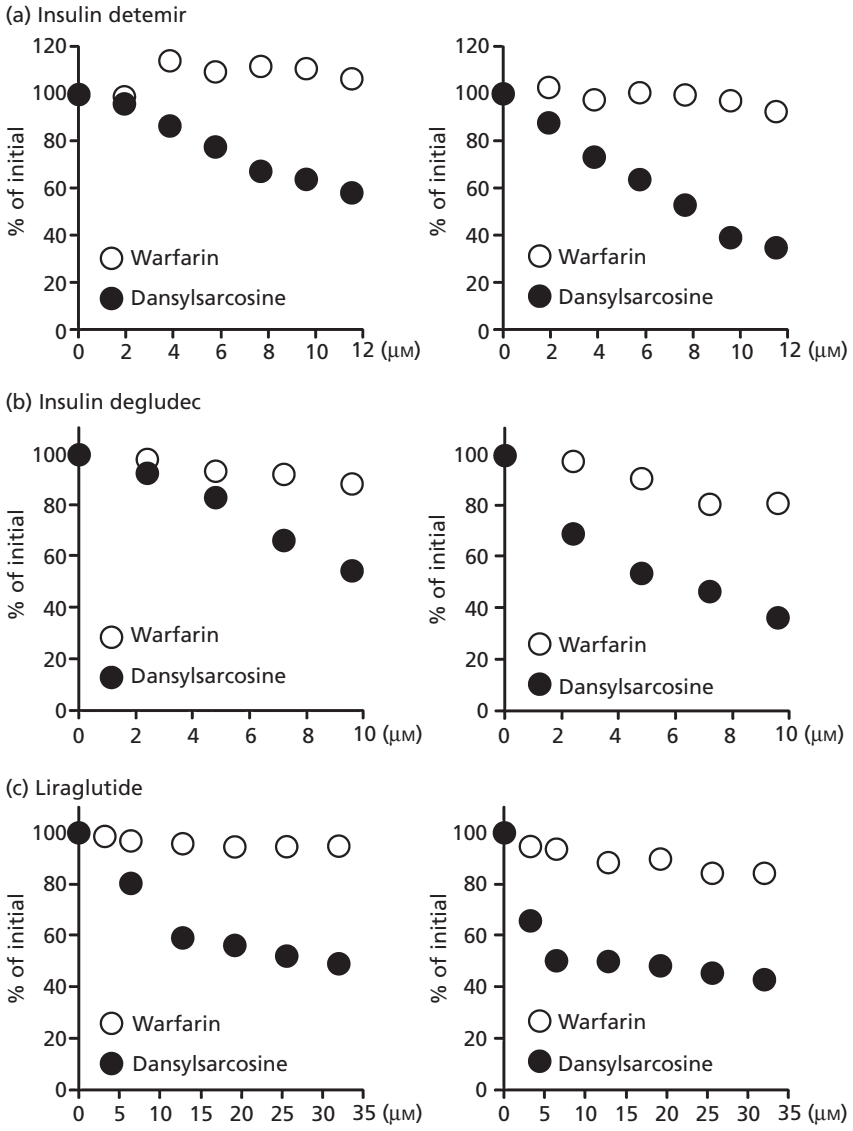

Figure 3 Displacement experiments in human serum albumin monomer (right panel) and human serum albumin dimer (left panel) for warfarin potassium (open circles) and dansylsarcosine (closed circles) by (a) insulin detemir, (b) insulin degludec and (c) liraglutide. Displacement experiments for warfarin potassium and dansylsarcosine by insulin analogues and glucagon-like peptide-1 (GLP-1) receptor agonist were performed by recording the fluorescence of a solution containing 2 μM warfarin potassium (or 2 μM dansylsarcosine) and 2 μM human serum albumin monomer (or 1 μM human serum albumin dimer). The excitation wavelengths for warfarin potassium and dansylsarcosine were 320 and 350 nm, respectively. The emission spectra for warfarin potassium and dansylsarcosine were recorded in the range of 350–450 nm and 400–600 nm, respectively.

STZ-induced diabetic mellitus animals were widely used in experiments that evaluate efficacy of antidiabetic agents. Thus, we evaluated the blood retention property of HSA dimer in STZ-induced diabetic nephropathy model rats. The $t_{1/2}$ for the HSA dimer was 1.5 times longer than that of the HSA monomer in STZ-induced diabetic nephropathy rat (Figure 2, Table 1). In addition, the mean resident time for HSA dimer was significantly longer than that of the HSA monomer (Table 1), which implied that HSA dimer had a prolonged retention in blood circulation as compared with HSA monomer. It is well accepted that glucose toxicity induces barrier dysfunction via direct targeting of the exchange microvascular endothelium that ultimately lead to increased extravasation of plasma fluid and proteins, including albumin.[14] In fact, leakage of exogenously infused macromolecules, such as albumin and dextran, from vascular space to extravascular space were detected in the diabetic model rats.[15,16] In a previous study, Matsushita *et al.* reported that the $t_{1/2}$ of genetically fused HSA dimer was prolonged by 1.1 times due to a decrease in the vascular

permeability of the HSA dimer compared with the HSA monomer in carrageenin-air-pouch rats.[7] Therefore, increasing the molecular size of HSA via chemical dimerization is thought to have retarded HSA dimer extravasation through the vascular endothelium, resulting in longer plasma retention in this study. In fact, in the diabetic model rats, the distribution of HSA dimer in muscle ($10.5 \pm 0.1\%$ of dose) was also less than HSA monomer ($14.8 \pm 2.1\%$ of dose) (Figure 2b).

The glomerular biological membrane has properties that do not allow larger proteins such as albumin to be filtered. This restriction can be explained by several mechanisms, including charge repulsion in the glomerular basement membrane and barrier.[17] As diabetes progresses, functional or structural glomerular alterations eventually will occur. Satoh *et al*. reported the increased glomerular permeability of 40 kDa rhodamine-labeled dextran in STZ-induced diabetic rats compared with normal rats.[18] Therefore, it is anticipated that increasing the molecular size of HSA by dimerization might prevent filtration by the renal glomerulus, results in a longer lifetime in blood circulation. However, there was no significant difference in distribution in kidney between HSA monomer and dimer in this study (Figure 2b). Diabetic nephropathy is classified into five stages (stages I–V) depending on the progression of renal failure, massive albuminuria can be observed in advanced diabetes (stages III–V).[19] To evaluate the progression of diabetic nephropathy, we determine the urinary protein concentration in the rat model of diabetic nephropathy at 14 days after STZ administration. The urinary protein in the diabetes model rat was 12.7 ± 3.5 mg/day, which was much less than that of the nephritic rats induced by doxorubicin (213 ± 28 mg/day) but was close to that of normal rats (8.0 ± 3.4 mg/day).[9] In addition, the urinary excretion of the HSA monomer and dimer in STZ-induced diabetic nephropathy rat were similar to those in normal rat.[9] Furthermore, although glomerulus damage was observed in the STZ-induced diabetic nephropathy model rats from the PAS staining (Figure 1g), Scr and BUN concentrations in the STZ-induced diabetic nephropathy model rats were at about the same level as those of the control rats (Figure 1e and 1f). These results suggested that the extent of diabetic nephropathy did not progress to end-stage renal failure in the diabetic nephropathy rat used in this study. Thus, this may explain no difference in the distribution in kidney between HSA monomer and dimer.

Although ^{111}In-HSA dimer showed a similar tendency of tissue distribution with that of the ^{111}In-HSA monomer, the extent of accumulation of the HSA dimer in the liver was much greater than that of ^{111}In-HSA monomer (Figure 2b). Rennen *et al*. previously reported that a rapid blood clearance with a predominant uptake by the liver was found for the largest proteins, while a relatively slow blood clearance with relatively moderate uptake by the liver was seen for proteins of intermediate size.[20] Therefore, higher hepatic distribution of HSA dimer than HSA monomer could be due to the increased molecular size of HSA via chemical dimerization. This is also supported by our previous pharmacokinetic study using normal rats that showed same tendency as in this study.[9]

To function as a retaining agent of fatty acid-conjugated insulin analogues and GLP-1 receptor agonist, HSA dimer must preserve the binding capacity of native HSA. These drugs can reversibly bind albumin through the long-chain fatty acid (myristyl, hexadecandioyl and palmitoyl fatty acid for insulin detemir, insulin degludec and liraglutide, respectively) that attached via a spacer to an amino acid in insulin or mutated GLP-1.[2,3] In this study, the fluorescent probe displacement experiment results for HSA monomer and dimer were similar, where insulin analogues and GLP-1 receptor agonist displaced DNSS but not WF in a concentration-dependent manner (Figure 3).

X-ray crystallographic studies revealed that HSA has seven fatty acid binding sites.[21] In addition, the fatty acid binding affinity (high/low) of each site has been also identified with ^{13}C nuclear magnetic resonance spectroscopy[22,23] and site-directed mutagenesis of HSA.[24] These studies identified that fatty acid binding sites 3 and 4, which correspond to a major drug binding site II, are high-affinity fatty acid binding sites. On the other hand, site I is also found to be a location for another one of the fatty acid binding sites. Based on molecular dynamics simulations reported by Fujiwara *et al.*, fatty acids preferentially bound to high-affinity fatty acid binding sites 2, 4 and 5 in the presence of WF.[25,26] This may explain insulin analogues and GLP-1 receptor agonist would only displace DNSS. These results suggest that the binding site and binding capacity of HSA monomer were preserved in the HSA dimer. In fact, a previous study showed that the binding constants of WF and diazepam for the chemically linked HSA dimer were similar to those for the HSA monomer.[8]

Although this study demonstrated the utility of HSA dimer as a plasma–retaining agent for the fatty acid-conjugated antidiabetic drugs, some limitations must also be taken into account. The dose used in this study (1 mg/kg) was less than a pharmacological dose in clinical situation. Therefore, a pharmacokinetic examination of HSA monomer and dimer at pharmacological doses should be a subject of future investigation. Another major point of experimental limitation noted in this study is that there was no direct evidence that HSA dimer can work as a plasma-retaining agent for the fatty acid-conjugated antidiabetic drugs *in vivo*. In the presence of endogenous albumin *in vivo*, these fatty acid-conjugated antidiabetic drugs would interact with not only HSA dimer but also endogenous albumin (monomer), and possibly other fatty acid binding

proteins, too. Therefore, it is too difficult to obtain direct evidence showing HSA dimer can work as a plasma-retaining agent *in vivo*. However, this could be a subject of further study in future.

Conclusion

The BMH-linked HSA dimer shows potential for use as a plasma-retaining agent for the fatty acid-conjugated antidiabetic drugs due to its favourable pharmacokinetic properties. Furthermore, the chemical and genetic HSA dimers have been reported to have potential for use as a drug carrier, novel plasma expander and artificial oxygen carrier because of their physicochemical properties and pharmacokinetic properties.[7,9,27,28] Since there are a number of HSA-conjugated drugs that have already been marketed or in advanced clinical trials,[29,30] HSA dimer is expected to further expand the application possibility for use in various clinical conditions.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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