=Supporting information=

Hemoglobin–albumin cluster: physiological responses after exchange transfusion into rats and blood circulation persistence in dogs

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Methods

Compatibility with canine blood in vitro

Canine whole blood was withdrawn from beagle dogs and was stored in a blood collection tube (Venoject II, EDTA-2Na; Terumo Corp.). Then, after the Hb-HSA$_3$ solution was added to the blood ([Hb-HSA$_3$] = 0, 10, 20 and 40 vol%, total volume 0.4 mL each) in a plastic microtube, the individual sample was mixed by inverting. The blood cell number of each sample was found using a veterinary use hematology analyzer (Celltac Alpha MEK-6450; Nihon Koden Corp.). The results are represented as percent ratios against the blood cell number of samples without dilution (0 vol% Hb-HSA$_3$). As control groups, the blood suspensions mixed with HSA (5 g/dL) ([HSA] = 10, 20 and 40 vol%) were also measured. Data are shown as mean ± standard error (SEM) (n = 3).

Canine whole blood was withdrawn from beagle dogs using syringe pretreated with 3.2% citric acid sodium salt. After the Hb-HSA$_3$ solution was added to the blood ([Hb-HSA$_3$] = 0, 10, 20 and 40 vol%, total volume 1.2 mL each) in a plastic microtube, the individual sample was mixed by inverting. These samples were centrifuged (2800 × g) for 15 min at 4 °C. The supernatant (0.6 mL) was transferred to a separation tube (S-1; BML Inc.) and was immediately frozen at −80 °C. The determination of prothrombin time (PT) was conducted by FUJIFILM Monolith Co., Ltd. (Tokyo, Japan). As a control group, blood suspensions diluted with HSA (5 g/dL) (10, 20 and 40 vol%) were also measured. Data are shown as mean ± SEM (n = 3).

All animal handling and care were done in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). The protocol details were approved by the Animal Care and Use Committee of Kyoritsu Seiyaku Corp.

Results

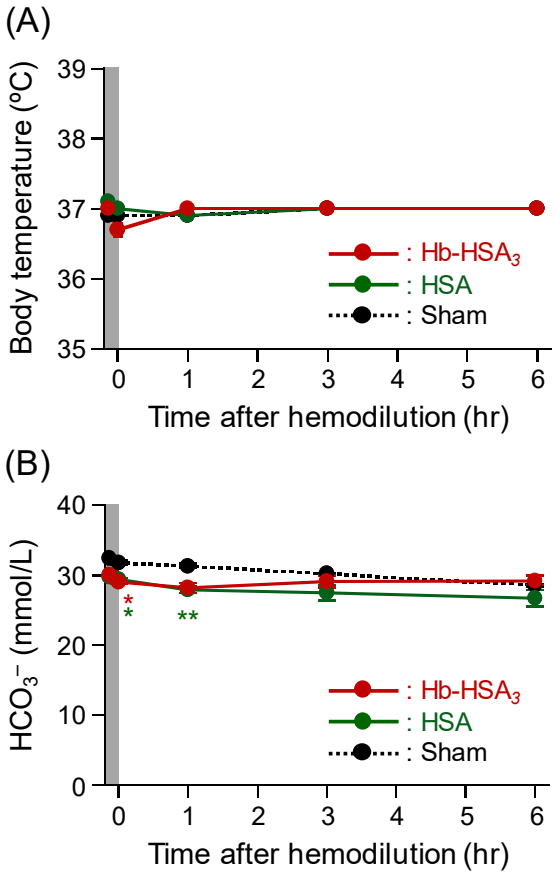

Figure S1. Change of body temperature and HCO_3^-. Time course of (A) body temperature and (B) HCO_3^- of anesthetized rats after 20% exchange transfusion with Hb-HSA$_3$ and HSA solution. Each datum represents mean ± SEM (n = 3). *p < .05 vs. sham group, **p < .01 vs. sham group.

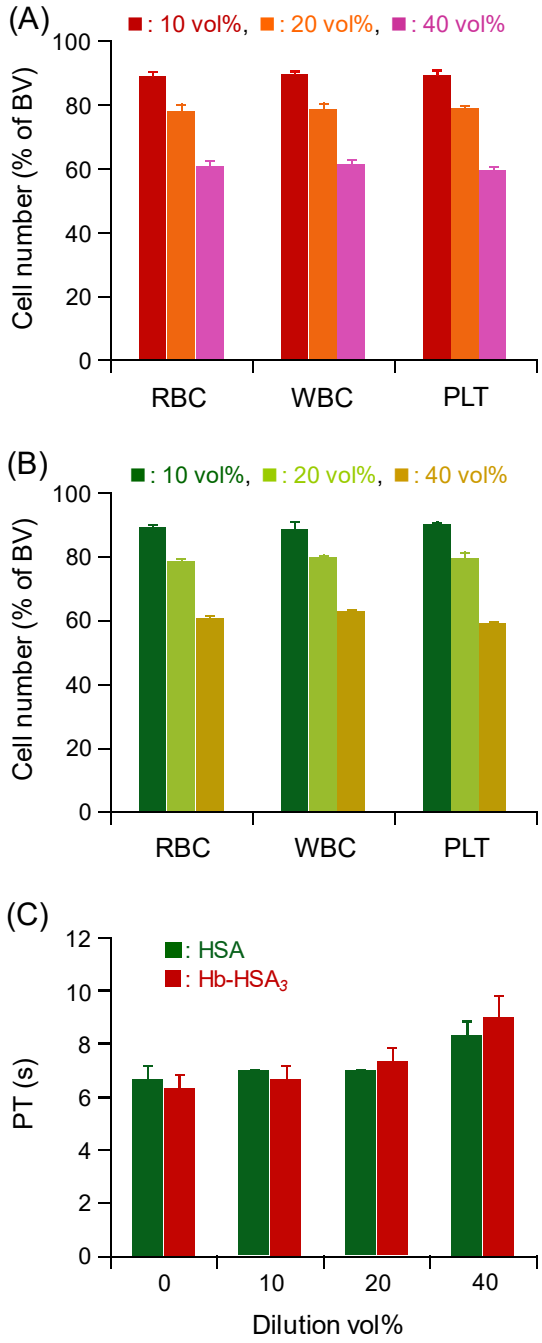

Figure S2. Compatibility with canine blood. (A) Blood cell (RBC, WBC, PLT) numbers in canine blood/Hb-HSA$_3$ mixture suspension ([Hb-HSA$_3$] = 10, 20 and 40 vol%). Basal values (BVs) were 801 ± 14 × 10^4 cells/μL in the RBC group, 94 ± 5 × 10^2 cells/μL in the WBC group and 39 ± 1 × 10^4 cells/μL in the PLT group. Each datum represents mean ± SEM (n = 3). (B) Blood cell (RBC, WBC, PLT) numbers in canine blood/HSA mixture suspension ([HSA] = 10, 20 and 40 vol%). Basal values (BVs) were 810 ± 17 × 10^4 cells/μL in the RBC group, 99 ± 5 × 10^2 cells/μL in the WBC group and 40 ± 1 × 10^4 cells/μL in the PLT group. Each datum represents mean ± SEM (n = 3). (C) PT values of canine blood samples after mixing with Hb-HSA$_3$ and HSA solution (10, 20 and 40 vol%). Each datum represents mean ± SEM (n = 3).

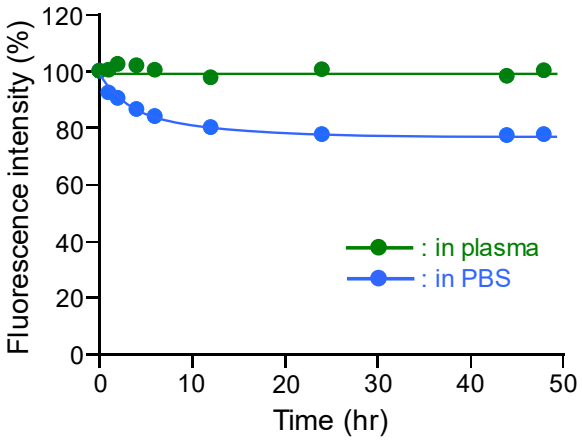

Figure S3. Stability of Hb-HSA$_3$(Cy5.5). Time courses of fluorescence intensities of Hb-HSA$_3$(Cy5.5) (λ_{em}: 707 nm) in plasma and PBS at 37 °C.

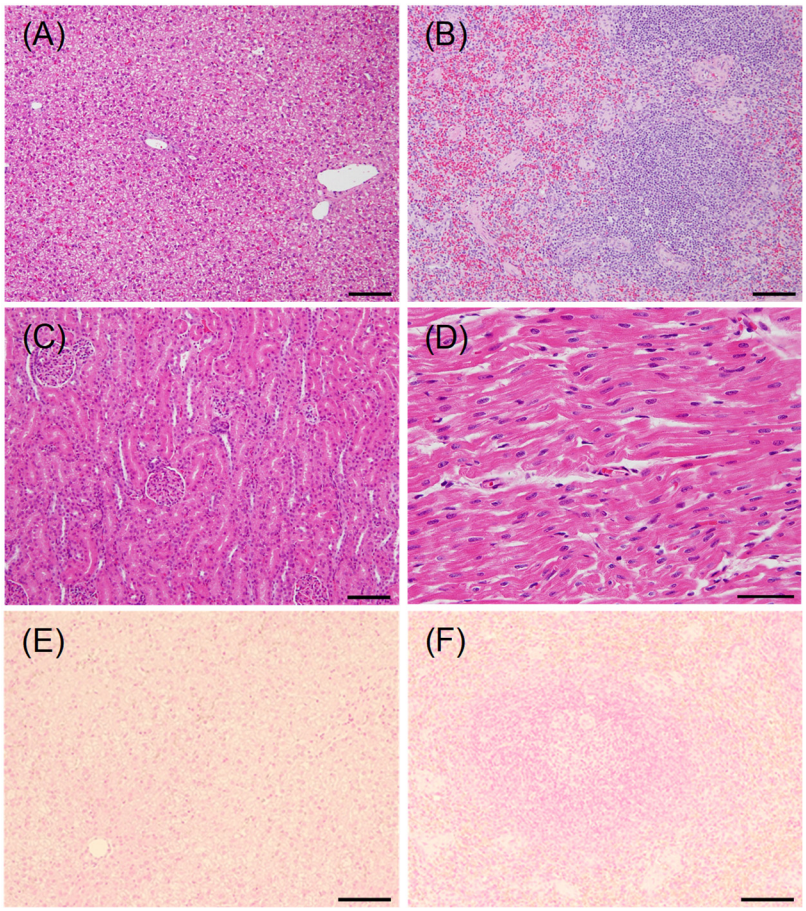

Figure S4. Histopathologic observations. Microscopic observations of stained specimens of vital organs recovered from dog after intravenous administration of Hb-HSA$_3$ solution. Hematoxylin–eosin (HE) stain: (A) liver, (B) spleen, (C) kidney and (D) heart. Belin blue stain: (E) liver and (F) spleen. Scale bar: 100 μm (A, B, C, E, F), 50 μm (D).

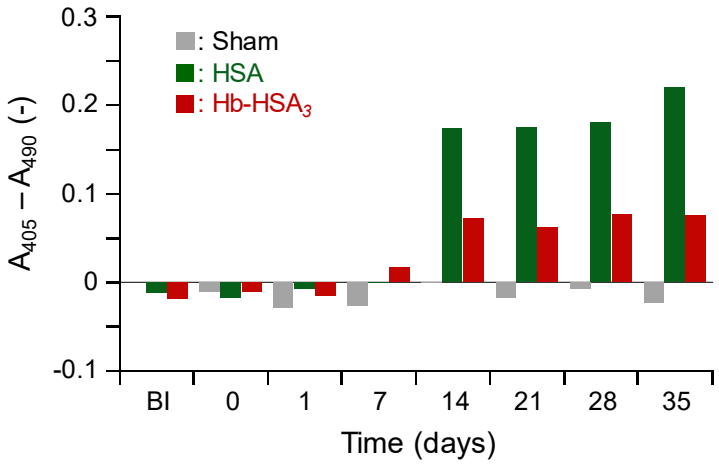

Figure S5. The generation of anti-HSA antibody in canine plasma. Time courses of differential absorption ($A_{405} - A_{490}$) of the specimen, which is proportional to concentration of anti-HSA antibody in blood plasma after intravenous administration of Hb-HSA$_3$ and HSA solution in dogs. The dose rate was 10% of the total blood volume. BI: just before infusion, 0: immediately after infusion.