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Supporting Information

Dual delivery of carbon monoxide and doxorubicin using haemoglobin-albumin cluster: Proof of concept for well-tolerated cancer therapy

Chihiro Ito,[a] Kazuaki Taguchi,[a*] Taiga Yamada,[b] Kengo Hanaya,[c] Yuki Enoki,[a] Takeshi Sugai,[c] Teruyuki Komatsu [b] and Kazuaki Matsumoto [a]

[a] Division of Pharmacodynamics, Faculty of Pharmacy, Keio University, Tokyo, Japan
[b] Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, Tokyo, Japan
[c] Division of Organic and Biocatalytic Chemistry, Faculty of Pharmacy, Keio University, Tokyo, Japan

* Corresponding author:
Kazuaki Taguchi, Ph.D.
e-mail: taguchi-kz@keio.jp

Experimental

Dynamic laser scattering (DLS) measurement

The particle size and polydispersity index were measured using Mobius (Wyatt Technology, CA, USA). Zeta potential was recorded using an ELSZ2KOP zeta potential analyser (Otsuka Electronics, Osaka, Japan). Samples were diluted in PBS and filtered through the syringe filter (0.22 μm) prior to measurement. All measurements were performed at 25 °C.

Circular dichroism (CD)

CD spectra were measured using a spectrometer (J-1100, JASCO, Tokyo, Japan). Each sample was diluted in PBS (final concentration: [haemoglobin] = 0.2 μM), and quartz cuvettes of 10 mm thickness were used for 200-260 nm measurements.

Size exclusion chromatography (SEC)

SEC system consisted of a column (YMC-Pack Diol-300, 8.0 mm × 300 mm i.d., 5 μm, YMC Co. Ltd., Kyoto, Japan), UV detector (SPD-20A, Shimadzu Corp., Kyoto, Japan), Japan), fluorescence detector (RF-10A XL, Shimadzu Corp., Kyoto, Japan), and pump (LC-20AD, Shimadzu Corp., Kyoto, Japan). The mobile phase consisted of 50 mM phosphate buffer (pH 7.4). The flow rate and column oven temperature were set to 0.5 mL/min and 25 °C, respectively. The UV detector wavelength was set at 280 nm for the protein, and the fluorescent detector wavelengths were 495 and 550 nm for DOX.

Native-PAGE and isoelectric focusing

Native-PAGE and isoelectric focusing were performed using a 6% polyacrylamide gel (Fujifilm Wako Pure Chemical, Osaka, Japan) and a pH 3–10 IEF gradient gel (Thermo Fischer Scientific, MA, USA), respectively. Gels were stained with Coomassie Blue R-250 (Nacalai Tesque, Kyoto, Japan), and images were obtained using an Amersham Imager 600 (Cytiva, USA). Samples for these measurements were adjusted at a concentration of [haemoglobin]=4 μM.

Cell source

Murine colon adenocarcinoma cells (Colon-26 cells), human breast cancer cells (MDA-MB-231 cells), murine melanoma cells (B16F10 cells), human ovarian adenocarcinoma cells (SKOV-3 cells), and human gastric carcinoma cells (NCI-N87 cells) were purchased from ATCC (Manassas, VA, USA). Human pancreatic cancer cells (AsPC-1 cells) and rat cardiomyoblasts (H9c2 cells) were obtained from KAC Co., Ltd. (Kyoto, Japan). Human breast cancer cells (MCF-7 cells) were obtained from the Cell Resource Center for Biomedical Research, Tohoku University. Human leukaemia cells (K562 cells) were provided by Professor Sugimoto, Keio University.

Cell culture

Colon-26, NCI-N87, AsPC-1, and K562 cells were cultured in RPMI1640. MCF-7, MDA-MB-231, B16F10, and H9c2 cells were cultured in Dulbecco's modified Eagle medium. The SKOV-3 cells were cultured in McCoy's 5a medium. All media contained 10% foetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were maintained at 37 °C in 5% CO_2.

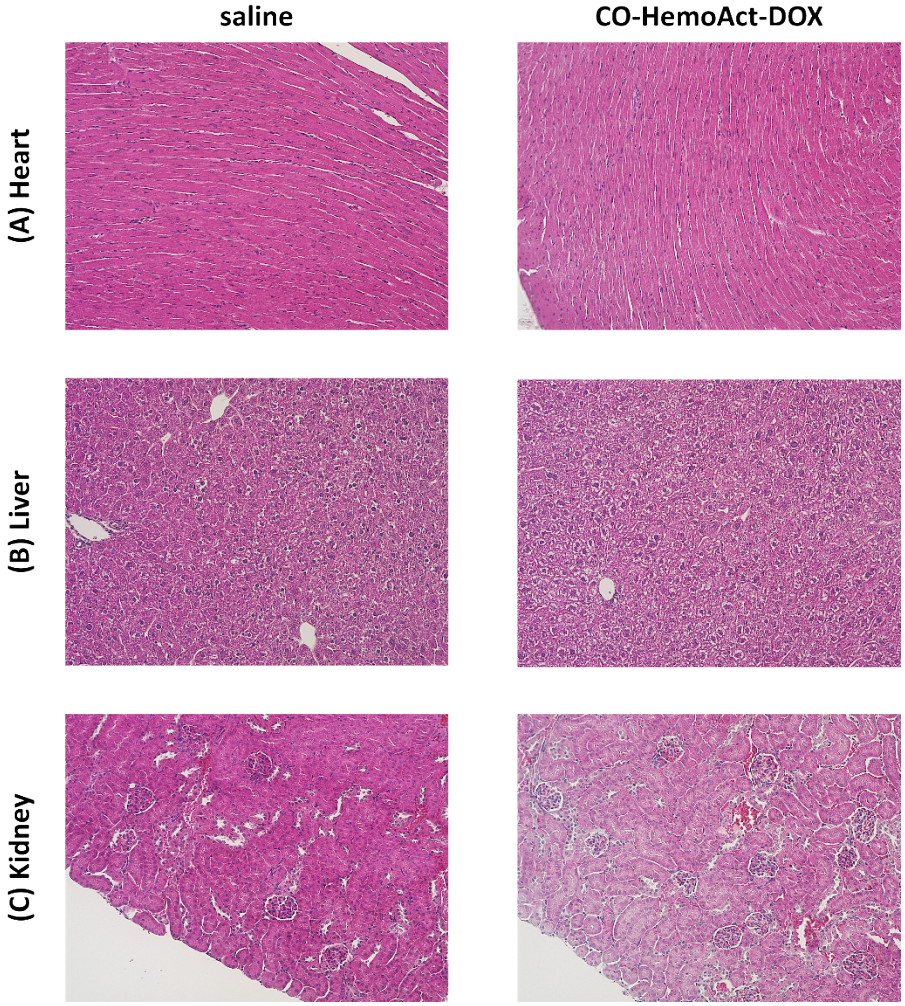

Figure S1. Representative images of (A) heart, (B) liver, and (C) kidney with haematoxylin and eosin after administration of CO-HemoAct-DOX. (200× magnification)

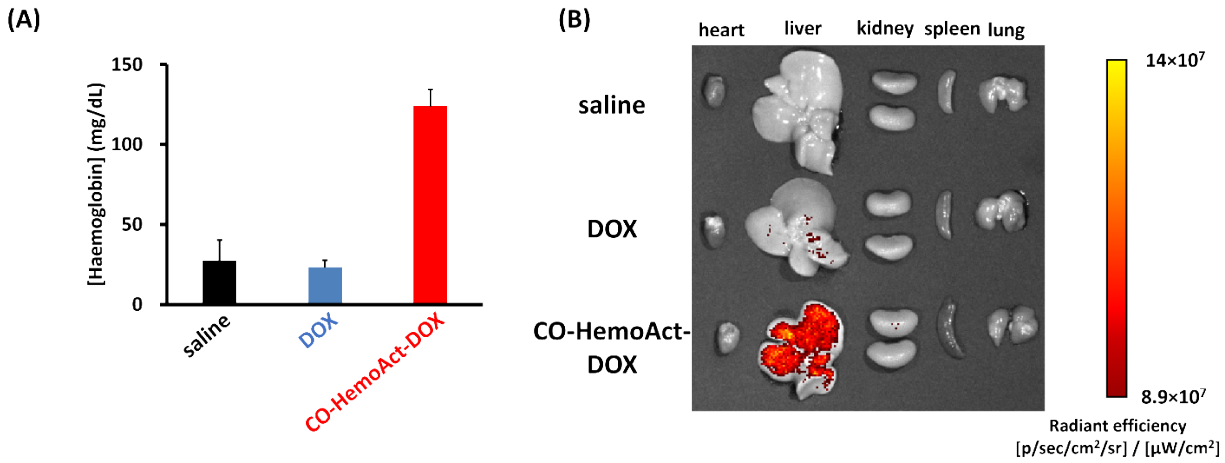

Figure S2. (A) Haemoglobin concentration in plasma collected from healthy mice at 24 h after saline, DOX, and CO-HemoAct-DOX administration. Each bar represents the mean ± S.D. (n =5). (B) *Ex vivo* imaging of DOX-derived fluorescence in organs collected from healthy mice administered either DOX or CO-HemoAct-DOX. Images were obtained 24 h after injection.

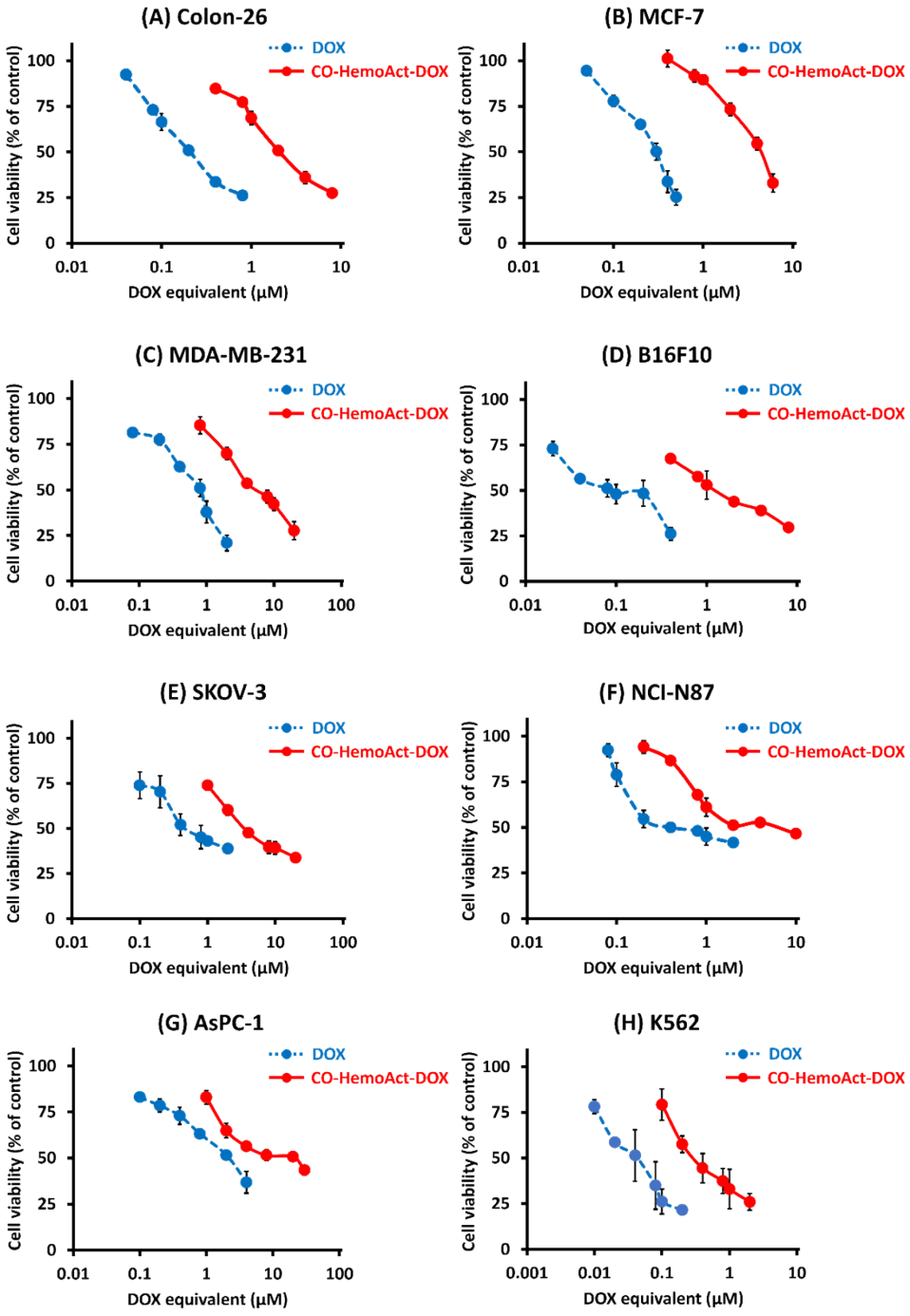

Figure S3. Cell viability of 2D-cultured (A) Colon-26 cells, (B) MCF-7 cells, (C) MDA-MB-231 cells, (D) B16F10 cells, (E) SKOV-3 cells, (F) NCI-N87 cells, (G) AsPC-1 cells, and (H) K562 cells at 48 h after treatment with DOX and CO-HemoAct-DOX. Data are represented as mean ± S.D. (n=3).

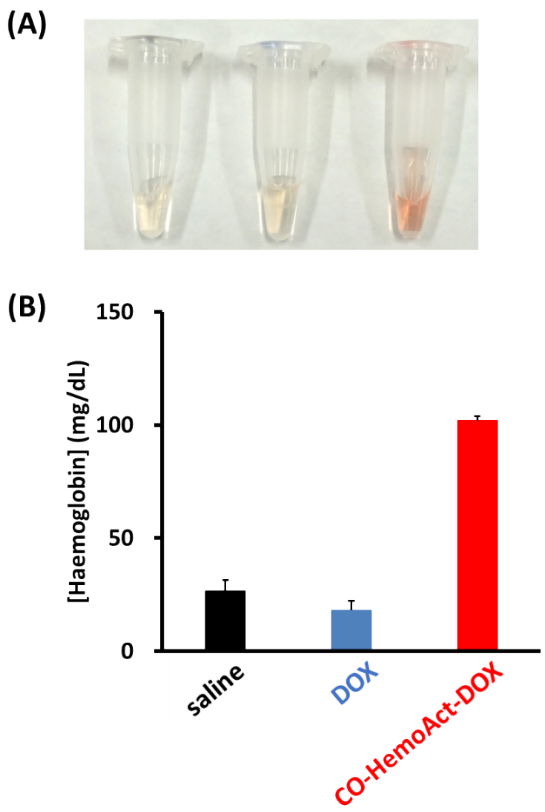

Figure S4. (A) The appearance of plasma collected from Colon-26 tumour-bearing mice at 24 h after saline, DOX, and CO-HemoAct-DOX administration. (B) Haemoglobin concentration in plasma collected from Colon-26 tumour-bearing mice at 24 h after saline, DOX, and CO-HemoAct-DOX administration. Each bar represents the mean ± S.D. (n=3).

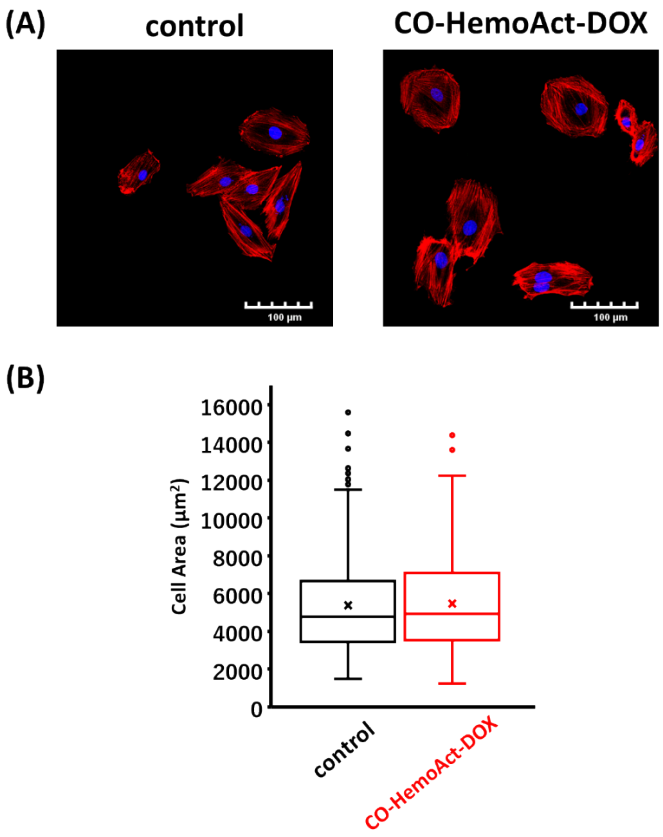

Figure S5. (A) Morphology and (B) cell area of H9c2 cell after CO-HemoAct-DOX treatment ([DOX]=1 µM). Scale bar: 100 µm. Data are represented as mean ± S.D. (n=3).