Supporting Information

A. Materials and Methods

Materials. Palmitoyl-oleoyl phosphatidylcholine (POPC), doxorubicin hydrochloride (DXR), dexamethasone (DEX) and 5-Fluorouracil (5-FU) were from Wako (Osaka, Japan). Trichostatin A (TSA) and zinc phthalocyanine (ZnPc) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Cis-diamineplatinum(II) dichloride (CDDP) was from SIGMA (St. Louis, MO). Dimethyl sulfoxide (DMSO) was obtained from Nacalai Tesque (Kyoto, Japan). Spectra/Por Dialysis Membranes (molecular weight cutoff 3,500 and 50,000) were from Spectrum Laboratories (Rancho Dominguez, CA). The NAP-5 Column was purchased from GE Healthcare UK Ltd. (Buckinghamshire, England). Native human HDL (nHDL) was from Biomedical Technologies, Inc. (Stoughton, MA).

Human apoA-I cDNA was provided by Dr. Akira Matsunaga at Fukuoka University.[1] The expression vector pCOLD I was purchased from TAKARA BIO INC. (Shiga, Japan). *E. coli* strain Origami2 was from Novagen (Madison, WI). Bacto™ Trypton and Yeast extract were from BD (Franklin Lakes, NJ). Nickel-chelate resin ProBOND was purchased from Invitrogen (Carlsbad, CA). *Ex Taq* DNA polymerase, restriction enzymes and T4 DNA ligase were purchased from TAKARA BIO INC. (Shiga, Japan). DH5α, Isopropyl β-thiogalactopyranoside (IPTG) and carbenicillin sodium salt were from Wako (Osaka, Japan).

Preparation of reconstituted nascent HDLs. The expression vector pGEX-2T containing the cDNA encoding the pre-propeptide form of human apoA-I[1] was first digested with *Bam*HI. The fragment containing the cDNA was cloned into the *Bam*HI site of the bacterial cloning vector pBluescript II KS(+) (Stratagene, La Jolla, CA, USA). The orientation of the cDNA transcripts was made in the same direction as the lacZ' transcripts. Amino-terminal 43 residue-deleted human apoA-I (ΔapoA-I) cDNA was synthesized by PCR using the pBluescript construct as the template and the primers 5'-taagaaggagatatacatatgctaaagctccttgacaac-3' (sense strand) and 5'-aattaaccctcactaaaggg-3' (anti-sense strand) to delete nucleotide sequences encoding the pre-pro region and NH_2-terminal 43 amino acid residues, and then was cloned into the *Nde*I and *Pst*I sites of the pCOLD I bacterial expression vector. Human apoA-I and ΔapoA-I were expressed in Origami2 according to the manufacturer's protocol, and

purified by nickel affinity chromatography under a denaturation condition.

Nascent HDLs were prepared according to the procedure described by Rogers et al. with minor modifications.[2] ApoA-I or ΔapoA-I in 4 M urea in phosphate-buffered saline (PBS) and POPC in 30 mg/ml sodium cholate in PBS were mixed at a 100:1 – 250:1 molar ratios of POPC to protein. The mixture was incubated overnight at room temperature and then dialyzed against PBS at 4°C for 24 h with two dialysate changes. The reconstituted nascent HDL solution was centrifuged at 16,500 *g* at 4°C for 10 min to remove debris. Nascent HDLs reconstituted with apoA-I and ΔapoA-I were designated as rHDL and rΔHDL, respectively. Their protein content was determined using D_c protein assay kit (BIO-RAD, Hercules, CA) in the presence of 0.1% Triton X-100.

Incorporation of doxorubicin (DXR) into nascent HDLs and native HDL. Each HDL (100 μg protein/mL) were loaded with DXR (400 μg/mL) by incubation at 37, 50 or 60°C for 0.5, 1 or 1.5 h. The reaction mixtures were passed through a NAP-5 gel filtration column equilibrated with 0.9% NaCl to remove free DXR and insoluble aggregates. The amount of DXR in HDLs was quantified spectrophotometrically at 485 nm in 2% SDS. Recovery rates of HDLs during the reactions were evaluated on a protein basis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or D_c protein assay. Since DXR interfered with the D_c protein assay to increase the apparent protein content, the contribution of DXR was subtracted from the D_c protein assay data.

To compare the DXR loading efficiency between nascent HDLs with native HDL, a previously reported procedure for native HDL was used.[3] Briefly, each HDL was mixed with DXR and gently shaken with a DeepWell Maximizer M•BR-022UP at 37°C for 4 h.

Incorporation of CDDP, TSA, ZnPc, DEX, 5-FU, and ATRA into rΔHDL. CDDP and 5-FU were dissolved in H_2O, and TSA, ZnPc, and ATRA were dissolved in DMSO. DEX was dissolved in ethanol or DMSO. Each drug solution (16 μL) was added to the rΔHDL solution, after which the mixtures were incubated at 50°C for 1 h or 60°C for 1.5 h. Then they were passed through the gel filtration column as described above.

Dynamic light scattering (DLS) analysis. Hydrodynamic diameters of DXR-free rΔHDLs in PBS and DXR-loaded rΔHDLs in 0.9% NaCl were determined with Nanotrac UPA-EX250 particle size analyzer (Nikkiso, Tokyo, Japan). The diameters of the other drug-loaded rΔHDLs were analyzed with ZetaSizer Nano Z (Malvern, Worcestershire, UK).

B. Supplementary Figures

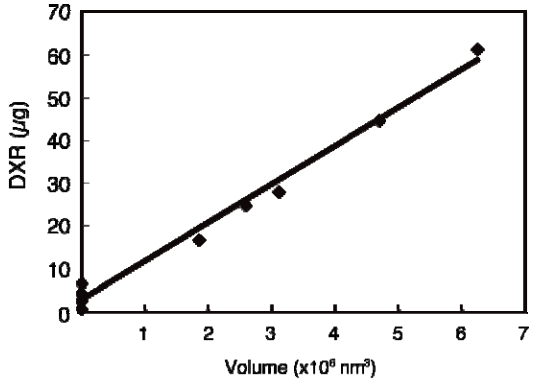

Fig. S1. Linear relationship between the DXR loading amount and the volume of DXR-loaded rΔHDL. The volume was calculated based on DLS data, and plotted against the DXR loading amount.

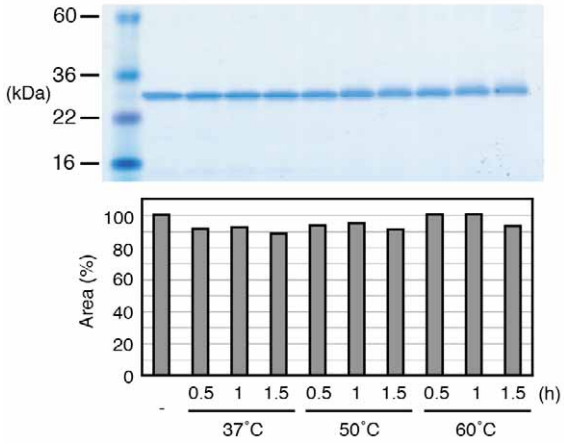

Fig. S2. The recovery rate of rΔHDL on a ΔapoA-I basis during DXR loading reaction. DXR-loaded rΔHDLs were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue staining (upper). Densitometric analysis of the blue gel bands was performed (below). All relative areas, corresponding to the recovery rates, were over 90%, relative to that of control, while smearing of the ΔapoA-I band occurred with increasing the temperature and time. Since no smearing was observed in the treatment of rΔHDL without DXR at 60°C for 1.5 h, some strong adsorption of DXR on ΔapoA-I would occur at higher temperature. Averages of data for two experiments were shown.

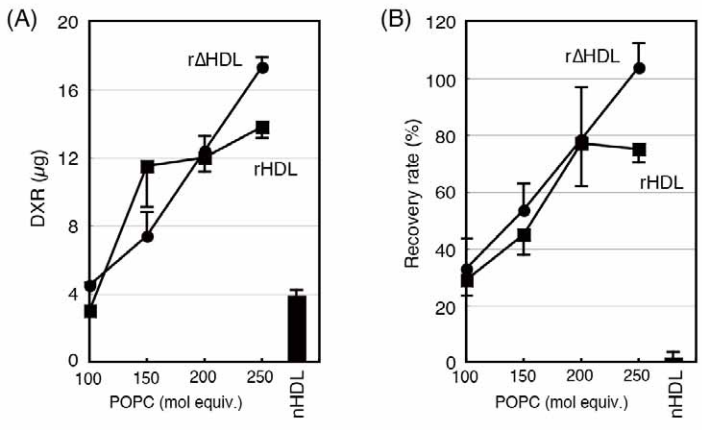

Fig. S3. Effects of the amount of POPC used for the nascent HDL preparation on the DXR loading. (A) The amount of DXR incorporated in rΔHDL and rHDL, which was prepared with apoA-I, and in nHDL. In both rΔHDL and rHDL, the amount of DXR was dependent on that of POPC used during their preparation. (B) The recovery rate of rΔHDL, rHDL, and nHDL on the basis of protein content determined by Lowry method. A POPC dose-dependent increase was observed for both rΔHDL and rHDL. In our experimental condition, nHDL showed poor stability. Taking into account the results in Fig. 2, it was also found that 250 molar equiv. of POPC is sufficient to prepare the thermally stable rΔHDLs. Averages of data for three experiments were shown.

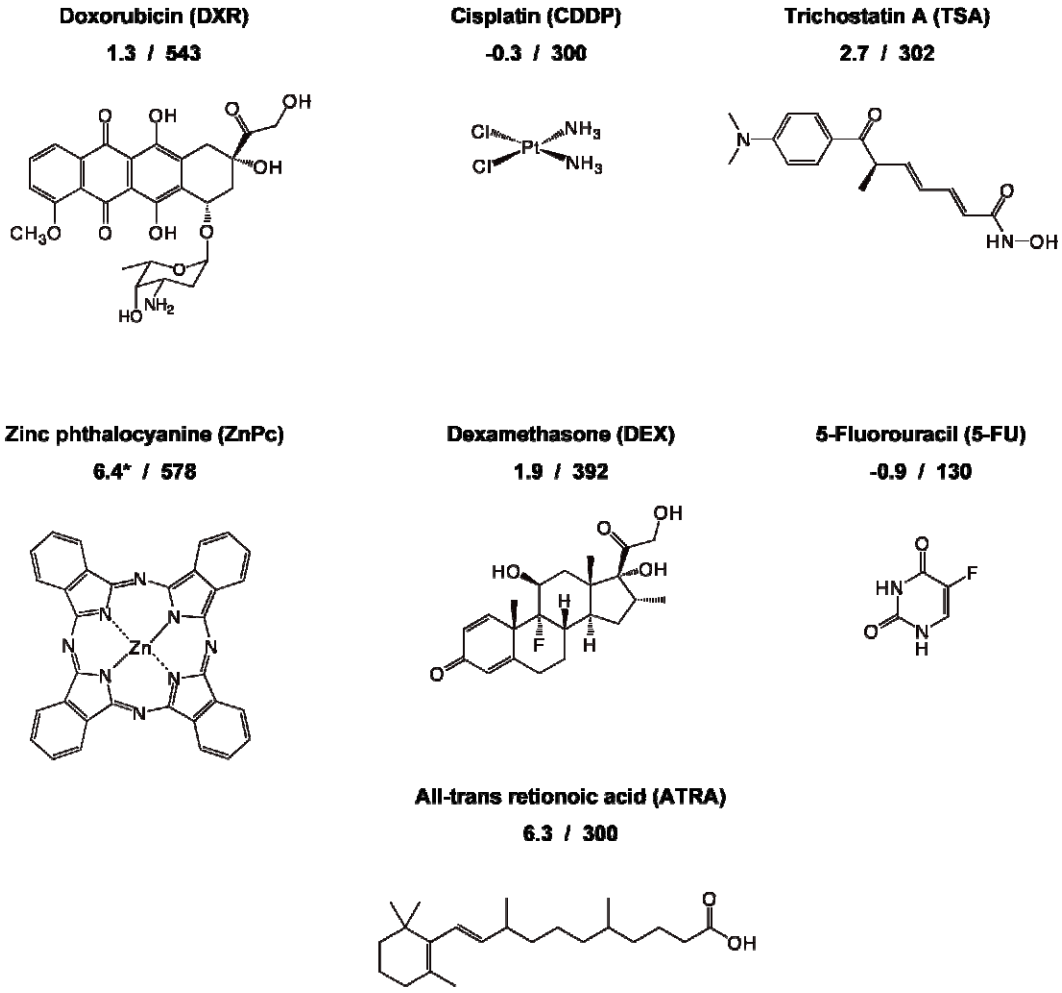

Fig. S4. Chemical structures of the drugs used in this study. The left and right numbers under the drug name denote the partition coefficient and molecular weight of the drugs, respectively, all of which were from PubChem (http://pubchem.ncbi.nlm.nih.gov/). * Data for zinc-free phthalocyanine.

C. References

1. H. Han, J. Sasaki, A. Matsunaga, H. Hakamata, W. Huang, M. Ageta, T. Taguchi, T. Koga, M. Kugi, S. Horiuchi and K. Arakawa, *Arterioscler. Thromb. Vasc. Biol.*, 1999, **19**, 1447-1455.
2. D. P. Rogers, C. G. Brouillette, J. A. Engler, S. W. Tendian, L. Roberts, V. K. Mishra, G. M. Anantharamaiah, S. Lund-Katz, M. C. Phillips and M. J. Ray, *Biochemistry*, 1997, **36**, 288-300.
3. A. Kader and A. Pater, *J. Control. Release*, 2002, **80**, 29-44.