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High density lipoprotein from patients with valvular heart disease uncouples endothelial nitric oxide synthase

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ABSTRACT

Normal high density lipoprotein (HDL) protects vascular function; however these protective effects of HDL may absent in valvular heart disease (VHD). Because vascular function plays an important role in maintaining the circulation post-cardiac surgery and some patients are difficult to stabilize, we hypothesized that a deleterious vascular effect of HDL may contribute to vascular dysfunction in VHD patients following surgery. HDL was isolated from age-match 28 healthy subjects and 84 patients with VHD and during cardiac surgery. HDL pro-inflammation index was measured and the effects of HDL on vasodilation, protein interaction, generation of nitric oxide (NO) and superoxide were determined. Patients with VHD received either simvastatin (20 mg/d) or routine medications, and endothelial effects of HDL were characterized. HDL inflammation index significantly increased in VHD patients and post-cardiac surgery. HDL from VHD patients and post-cardiac surgery significantly impaired endothelium-dependent vasodilation, inhibited both Akt and endothelial nitric oxide synthase (eNOS) phosphorylation at S1177, eNOS associated with heat shock protein 90 (HSP90), NO production and *increased eNOS phosphorylation at T495 and* superoxide generation. Simvastatin therapy partially reduced HDL inflammation index, improved the capacity of HDL to stimulate eNOS and Akt phosphorylation at S1177, eNOS associated with HSP90, NO production, *reduced eNOS phosphorylation at T495* and superoxide generation, and improved endothelium-dependent vasodilation. Our data demonstrated that HDL from VHD patients and cardiac surgery contributed to endothelial dysfunction through uncoupling of eNOS. This deleterious effect can be reversed by simvastatin, which improves the vasoprotective effects of HDL. Targeting HDL may be a therapeutic strategy for maintaining vascular function and improving the outcomes post-cardiac surgery.

1. Introduction

Normal high density lipoprotein (HDL) is anti-inflammatory and vasoprotective [1]. Normal HDL can stimulate endothelial cells to generate nitric oxide (NO), exert antioxidant effects, promote angiogenesis and inhibit low density lipoprotein (LDL)-impaired endothelium-dependent vasodilation [2–6]. However, HDL may become pro-inflammatory instead of anti-inflammatory in disease states, such as in coronary artery disease (CAD), diabetes mellitus and chronic kidney dysfunction [7–13]. The HDL in these disease states has high pro-inflammatory index, increased oxidant accumulation and promotes the formation of LDL derived oxidized lipids[4]. Thus, the vascular effects of HDL can be highly heterogeneous [14,15].

Valvular heart disease (VHD) is one of the major heart diseases in the world [16,17]. Most VHD needs cardiac surgery for repair or require replacement under cardiopulmonary bypass. However, cardiopulmonary bypass surgery significantly disturbs normal physiology and leads to unstable circulation post-cardiac surgery [18,19]. Because vascular function plays an important role in maintaining the circulation post-cardiac surgery and some patients are difficult to stabilize [20], we hypothesized that a deleterious vascular effect of HDL may contribute to vascular dysfunction in VHD patients following surgery.

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We recently reported a clinical trial that perioperative simvastatin therapy could reduce myocardial injury, inflammatory response and the requirement of inotropic in patients undergoing noncoronary artery cardiac surgery [21]. Fogelman et al. have showed that simvastatin could improve HDL function in CAD [8]. Whether simvastatin affects the HDL function in VHD and during cardiac surgery to reduce myocardial injury and inflammatory response to protect vascular function and reduce the requirement of inotropic remains unknown. Therefore, the present study investigated if HDL from patients undergoing VHD and cardiac surgery produced vascular dysfunction, and if simvastatin would reverse this deleterious effect of HDL in a randomized controlled clinical trial.

2. Method and methods

2.1. Study population and design

Patients with VHD were diagnosed by echocardiography. Rheumatic heart disease (RHD) was diagnosed according to the classification criteria of Jones [22]. Other valvular heart diseases (OVHDs) include degenerated heart disease, mitral valve prolapse, and congenital valve malformation. Patients with coronary heart disease, diabetes, infectious disease, severe trauma and suffering surgery in recent 3 months were excluded. All subjects were above 18 years old. This study was approved by The First Affiliated Hospital, Sun Yat-sen University Ethics Review Board. Informed consent was obtained from all subjects enrolled in this study. The clinical characteristics of the population studied were summarized in Table 1.

Another group of VHD patients was selected from our previous simvastatin clinical trial (Clinical Trial Registration: http://www.clinicaltrials.gov, number NCT01178710) [21]. Briefly, *the patients were randomly assigned to either the simvastatin group or control group by a random number produced by a computer*. Simvastatin (20 mg) was administered to the elective cardiac valvular surgical patients every day for 5–7 days preoperatively, but not the day of surgery in the simvastatin group. Then simvastatin was re-administered at the second day postoperatively. The control group was administered all of the same routine medications as the simvastatin group, such as Digoxin, Furosemide, without simvastatin therapy as described previously [21]. The clinical characteristics of the population studied were summarized in Table 2.

2.2. Isolation of HDL and measurement of its pro-inflammatory state

Blood was collected from VHD patients before and at the 6th, 12th, 24th, and 48th hour after surgery and healthy subjects respectively. Plasma was isolated and stored in −80 °C until assay and HDL isolation. HDL was isolated from plasma via sequential ultracentrifugation as previously described [23]. Pro-inflammatory HDL was determined by using a cell-free assay as previously described [24]. *Since HDL from one patient was not enough to finish one set of following experiments, plasma from several patients were combined to isolate HDL to perform one set of experiment. The pre-operative and post-operative HDL samples in each experiment were from the same patients, which were chosen prospectively. HDL from healthy subject was selected prospectively and randomly.*

Table 1
Clinical characteristics.

Variables	Healthy (n = 28)	VHD (n = 84)
Age (years)	47.9 ± 8.9 (32–65)	51.8 ± 12.3 (23–79)
Male/female	12/16	34/50
C reactive protein, μmol/L	1.31 ± 0.77	4.88 ± 5.79*
Total cholesterol, mmol/L	4.41 ± 0.73	4.29 ± 0.82*
Triglyceride, mmol/L	1.08 ± 0.38	1.03 ± 0.43
High density lipoprotein, mmol/L	1.27 ± 0.16	1.12 ± 0.25*
Low density lipoprotein, mmol/L	2.30 ± 0.47	2.26 ± 0.62
NYHA class (male/female)		
	0	7 (2/5)
II	0	22 (11/11)
≥III	0	55 (21/34)

NYHA: New York Heart Association, *: $p < 0.05$.

Table 2
Clinical characteristics.

Variables	Statins (−)	Statins (+)
Participants	41 (21–70)	49 (25–72)
Age (years)	47.2 ± 11.4	50.8 ± 10.1
Male/female	18/23	20/29
Hypertension	4 (10)	7 (14)
Diabetes mellitus	2 (5)	3 (6)
Body mass index, kg/m^2	21.1 ± 7.8	22.2 ± 5.5
Total cholesterol, mmol/L	4.24 ± 0.93	4.29 ± 0.67
Triglyceride, mmol/L	1.14 ± 0.33	1.09 ± 0.32
High density lipoprotein, mmol/L	1.20 ± 0.3	1.18 ± 0.37
Low density lipoprotein, mmol/L	2.32 ± 0.59	2.39 ± 0.56
PASP (preoperation), mmHg	44.0 ± 16.8	41.6 ± 15.6
Medications		
Diuretics	41 (100)	49 (100)
Digoxin	40 (98)	47 (96)

Values are n, mean ± SD, n (%). PASP: pulmonary arterial systolic pressure.

2.3. Vasodilation study

All animal experiments were approved by the ethics review board and animal research committee of The First Affiliated Hospital, Sun Yat-sen University. Eight-week-old male C57BL6 mice and endothelial nitric oxide synthase (eNOS)−/− mice were obtained from Jackson Laboratory (Bar Harbor, MA). Mice were anesthetized by pentobarbital (Sigma-Aldrich, St. Louis, MO) and aortas were isolated. Four 3 mm wide aortic rings were obtained and connected to an isometric force transducer (DMT620M, AD Instruments). Aortic rings were suspended in organ chambers filled with Krebs-solution. After a 30 min equilibration period in non-stretching condition, the pretension was increased stepwise to 5 mN and allowed to rest over a period of 60 min. After this, the rings were exposed to 60 mmol/L KCl at least three times until optimal tension was achieved. *Our preliminary study and other reports suggested that HDL (0.26 mmol/L, equal to 100 μg/mL) had significant effect on endothelial cells, we used this concentration in the present study* [25]. Then aortic rings were *pretreated* with HDL (0.26 mmol/L) from health or patients for 30 min. Thereafter, aortic rings were preconstricted with 10^{-6} mol/L noradrenaline (NA, Sigma-Aldrich, St. Louis, MO). Endothelium-dependent vasodilation was determined with acetylcholine (Ach: 10^{-8}–10^{-4} mol/L. Sigma-Aldrich, St. Louis, MO) and NG-nitro-L-arginine methylester (L-NAME, 100 μmol/L, Sigma-Aldrich, St. Louis, MO), respectively. Endothelium-independent vasodilation was studied in response to the nitrovasodilator sodium nitroprusside (SNP; 10^{-8}–10^{-4} mol/L, Sigma-Aldrich, St. Louis, MO). *Aortic rings were pretreated nothing as control.*

2.4. Western blot analysis

Human umbilical vein endothelial cells (HUVECs, ScienCell Research Laboratories, San Diego, CA) at passages 4–6 were cultured in endothelial cell medium (ScienCell) supplemented with 5% fetal bovine serum (FBS), 1% growth factors, and 1% penicillin/streptomycin. After serum starvation with 0.5% FBS for 24 h, cells were treated with isolated HDL (0.26 mmol/L) from VHD patients or healthy subjects for 30 min. Cellular proteins were harvested and immunoblotting performed as described previously [26]. Antibodies of Akt, phosphorylation of Akt, and phosphorylation of eNOS at Ser1177 *and at T495* were purchased from Cell Signaling Technology (Danvers, MA). Anti-eNOS antibody was

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