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# HDL mimetic peptide CER-522 treatment regresses left ventricular diastolic dysfunction in cholesterol-fed rabbits☆

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ABSTRACT

*Objectives:* High-density lipoprotein (HDL) infusions induce rapid improvement of experimental atherosclerosis in rabbits but their effect on ventricular function remains unknown. We aimed to evaluate the effects of the HDL mimetic peptide CER-522 on left ventricular diastolic dysfunction (LVDD).
*Methods:* Rabbits were fed with a cholesterol- and vitamin $D_2$-enriched diet until mild aortic valve stenosis and hypercholesterolemia-induced LV hypertrophy and LVDD developed. Animals then received saline or 10 or 30 mg/kg CER-522 infusions 6 times over 2 weeks. We performed serial echocardiograms and LV histology to evaluate the effects of CER-522 therapy on LVDD.
*Results:* LVDD was reduced by CER-522 as shown by multiple parameters including early filling mitral deceleration time, deceleration rate, Em/Am ratio, E/Em ratio, pulmonary venous velocities, and LVDD score. These findings were associated with reduced macrophages (RAM-11 positive cells) in the pericoronary area and LV, and decreased levels of apoptotic cardiomyocytes in CER-522-treated rabbits. CER-522 treatment also resulted in decreased atheromatous plaques and internal elastic lamina area in coronary arteries.
*Conclusions:* CER-522 improves LVDD in rabbits, with reductions of LV macrophage accumulation, cardiomyocyte apoptosis, coronary atherosclerosis and remodelling.



## 1. Introduction

Left ventricular diastolic dysfunction (LVDD) is present in 11%–35% of the general population [1–4] and is associated with about half of the cases of heart failure [5–8]. As the incidence of LVDD increases with age [4], its prevalence is predicted to increase further in the next decades. LVDD is defined as impaired left ventricular filling due to abnormalities of relaxation and/or compliance, regardless of systolic function [9]. The most common etiologies of LVDD are arterial hypertension, LV hypertrophy, myocardial ischemia and aortic valve stenosis. Until now, very few studies have explored the pathophysiological mechanisms involved in LVDD and no specific treatment is available. Given its increasing prevalence, there is considerable need for effective therapeutics directly targeting LVDD.

Epidemiologic data have demonstrated that plasma levels of high-density lipoprotein (HDL) cholesterol are inversely related to cardiovascular risk [10]. In the specific case of LVDD, prospective clinical studies have suggested that a low HDL-cholesterol level is an independent predictor of LVDD in hypertensive patients [11,12] as well as in those with metabolic syndrome [13]. We have previously observed that rabbits fed with a cholesterol-enriched diet supplemented with vitamin $D_2$ develop both LVDD (unpublished data) and mild aortic valve stenosis [14]. Although infusions of reconstituted HDL have been shown to induce regression of mild aortic valve stenosis [14,15], their effect on LVDD is not known. The present study aimed to investigate the effects of CER-522, a new HDL mimetic composed of an apoA-I peptide analogue complexed with phospholipids, on LVDD in a cholesterol-fed rabbit model.

*Abbreviations:* ApoA-I, apolipoprotein A-I; EEL, external elastic lamina; HDL, high-density lipoprotein; IEL, internal elastic lamina; LV, left ventricle; LVDD, left ventricular diastolic dysfunction; TMF, transmitral flow; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

☆ All of the authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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## 2. Methods

### 2.1. Animal studies

Animal care and procedures complied with the Canadian Council on Animal Care guidelines and were approved by the Montreal Heart Institute ethics committee for animal research. The rabbit experimental model was developed as previously described [14]. Briefly, thirty-five male New Zealand White rabbits (2.7 kg, aged 12–13 weeks) were fed with a 0.5% cholesterol-enriched diet and vitamin $D_2$ (12,500 to 50,000 IU/day) in the drinking water until development of mild AVS, as defined by a ≥10% decrease in aortic valve area measured by echocardiography. LVDD was also classified according to previously published criteria [16–18] at that time, without differences between the 3 experimental groups described below ($P = 0.652$; Fig. 1). Following evaluation of LVDD, animals were fed with a standard diet (without cholesterol and vitamin $D_2$) to mimic cholesterol-lowering therapy, and were randomized to receive infusions of phosphate-buffered saline (control group, $n = 11$) or the HDL mimetic CER-522 at a dose of 10 mg/kg ($n = 12$) or 30 mg/kg ($n = 12$) 3 times per week for 2 weeks through the marginal ear vein. A normal diet group ($n = 14$), not exposed to a high-cholesterol diet during the initial phase and composed of rabbits of the same age, was also included in the study. Animals were killed 2 days after their last infusion (at day 14) by exsanguination under deep anaesthesia (ketamine 200 mg/kg, i.m.; xylazine 5 mg/kg, i.m.). The ventricles were removed for histological and cholesterol content analyses. Blood samples (2–5 mL) were obtained through the ear marginal vein at baseline and at days 0, 7 and 14 of treatment. Total cholesterol, HDL cholesterol, triglycerides and calcium levels were measured with an automated filter photometer system (Dimension RxL Max; Dade Behring, Deerfield, IL, USA). Also, at end of treatment (day 14), cholesterol profile was analyzed using fast protein liquid chromatography (FPLC)-size exclusion chromatography of lipoproteins on pooled plasma of each experimental group and lipoprotein particle profiles were measured by proton nuclear magnetic resonance (NMR) spectroscopy analysis on serum SST samples. Quantitative determination of plasma-oxidized LDL was assessed by a sandwich ELISA test (rabbit Ox-LDL ELISA kit ABIN416062, supplied by Antibodies-Online, Atlanta, GA, USA) (see Supplemental Data for details).

### 2.2. HDL mimetic CER-522

CER-522 (Cerenis Therapeutics, Ann Arbor, USA) is an engineered HDL mimetic comprised of a 22-amino acid amphipathic peptide with a non-natural amino acid, isonipecotic acid, at the C-terminal end (peptide sequence: H-Lys-Leu-Lys-Gln-Lys$^{5}$-Leu-Ala-Glu-Leu-Leu$^{10}$-Glu-Asn-Leu-Leu-Glu$^{15}$-Arg-Phe-Leu-Asp-Leu$^{20}$-Val-Inp$^{22}$-OH) and phospholipids. The peptide is formulated as a complex with dipalmitoyl phosphatidyl choline (48.5%), dipalmitoyl phosphatidyl glycerol (3%) and sphingomyelin (48.5%). When injected in animals, CER-522 behaves like pre-β HDL particle and mobilizes cholesterol (data not shown), similarly as the CER-001 HDL-mimetic lipoprotein particle engineered by the same company [19]. The peptide is capped at the N-terminal to ensure stability of the peptide helical structure and lipid binding compared to the peptides with loose terminal flanking end. Neither the peptide on its own nor the phospholipids have activity to mobilize cholesterol (see Supplemental Data for details).

### 2.3. Echocardiography

Examinations were carried out with a phased-array probe 10S (4.5–11.5 MHz) using a Vivid 7 Dimension system (GE Healthcare Ultrasound, Horten, Norway). Complete echocardiographic examinations (with Doppler) were performed at baseline and at days 0, 3, 7, 10 and 14 of the treatment to assess several LVDD parameters, aortic root strain index, LV mass and aortic valve area. Heart rate was monitored during all acquisitions and was similar between the 3 experimental groups during both diet period ($P = 0.076$) and treatment period (Supplemental Table 1). LVDD was classified using previously published criteria [16–18] (see Supplemental Data for details).

### 2.4. Histology and histomorphometry

Ventricles were harvested following sacrifice and processed as previously described [16]. Masson's trichrome stained sections were used to assess LV cavity area, LV wall area, LV fibrosis, LV lesion area and cardiomyocyte size. For coronary arteries, external and internal elastic lamina (EEL and IEL, respectively) and lumen areas were measured. Coronary plaque area was calculated as IEL area minus lumen area (see Supplemental Data for details).

### 2.5. Immunohistochemistry of LV sections

We assessed the levels of type I and III collagen on ventricular paraffin-embedded sections. We also evaluated macrophage infiltration (RAM-11 positive cells) (see Supplemental Data for details).

### 2.6. Apoptotic cell detection in LV sections

Apoptotic cells were detected by terminal deoxynucleotidyl transferase dUTP nick end-labelling (TUNEL) assay (see Supplemental Data for details).

### 2.7. In vitro smooth muscle cell proliferation assay

Effects of increasing CER-522 concentrations on MOVAS cells (mouse vascular smooth muscle cell (SMC) line, ATCC) proliferation

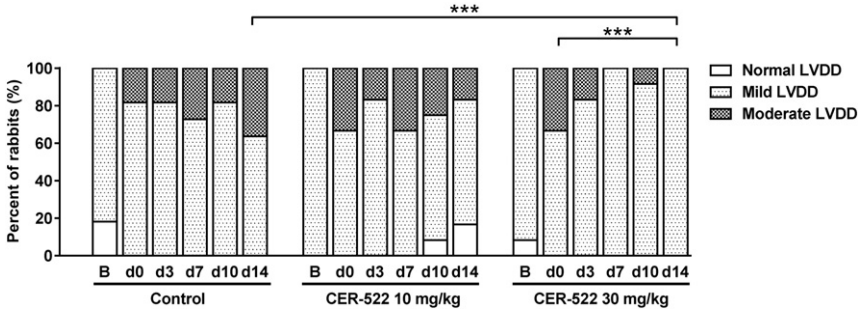


**Fig. 1.** Distribution of left ventricular diastolic dysfunction (LVDD) severity in rabbits at each time point. LVDD was aggravated from baseline (B) to the beginning of treatment (day (d) 0): percent of rabbits with normal and mild LVDD decreased whereas percent of rabbits with moderate LVDD increased without significant differences between the 3 experimental groups at day 0 ($P = 0.652$). During the treatment period, the percentage of rabbits with moderate LVDD decreased in the 30 mg/kg CER-522-treated group ($P < 0.001$) whereas it did not change in the control group ($P = 0.307$). $^{***}P < 0.001$.