Pitavastatin increases HDL2 cholesterol levels in chronic hemodialysis patients.

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(51st ERA-EDTA Congress , Amsterdam, June 1, 2014)

Aims

Chronic hemodialysis (HD) patients usually have lower plasma high-density lipoprotein (HDL)-cholesterol (C) and higher triglyceride (TG) levels than healthy subjects. Low HDL-C levels are associated with an increased risk of death in chronic HD patients. The aim of this study is to investigate the clinical characteristics and serum HDL subfractions of chronic HD patients, and investigate the effects of pitavastatin on HDL subfractions in chronic HD patients.

Subjects and methods

(1) Protocol 1
We established a single precipitation method for the measurement of HDL2 and HDL3 [J Lipid Res.49:1130-1136, 2008]. Using this simple assay, we examined HDL subfractions in forty-four HD patients and ten healthy controls.

(2) Protocol 2
Eleven chronic HD patients were treated with pitavastatin at 1 mg/day for 3 months. Plasma levels of total cholesterol (TC), TG, low-density lipoprotein (LDL)-C, small dense LDL-C (sd-LDL-C), HDL-C, HDL2-C, HDL3-C were measured at baseline and after 1 and 3 months of treatment with pitavastatin in the HD patients.

HDL subflactions

We recently established a simple method for assaying HDL2 and HDL3 by a single precipitation. Briefly, a precification regent (0.06 ml) containing 1,071 units/ml heparin, 500 mmol/l $MnCl_2$, and 12 mg/ml dextran sulfate (DS) was added to a serum (0.3 ml). The sample was incubated and centrifuged at 10,000 rpm for 10 min. Under this condition, all of the lipoproteins except HDL3 were precipitated with heparin-Mn-Ds, and HDL3 was isolated in the supernatant. HDL3-C was measured by a homogenous HDL-C assay (HDL-EX, Denka Seiken, Tokyo, Japan) in the supernatant, and HDL2-C was estimated by subtracting the HDL3-C from the direct HDL-C. The HDL3-C and HDL2-C values determined by the precipitation method were identical to those determined by ultracentrifugation, and there were excellent correlations (r>0.93) between these methods. The precipitation method and ultracentrifugation also proved to be highly correlated (r > 0.91) in the measurement of the apo A Ⅰ and apo A Ⅱ in the HDL subflactions. Total HDL was isolated by the classical precipitation method with heparin-Mn.

Table 1 Clinical characteristics.

	HD patients (n=44)	controls (n=10)
Male	26	3
Female	18	7
DM	21	
non DM	23	
Age (years)	68±13	49±11
HD duration (months)	68.1±70.8	

Table 2 Various serum parameters and lipids in HD patients.

(mg/dl)	HD (n=44)	controls (n=10)
CRP	0.34±0.58*	0.15±0.16
Triglyceride	130±63	171±89
Total-cholesterol	166.6±37.3**	204.3±42.4
LDL-cholesterol	86.6±31.2*	110.2±39.7
Sd-LDL-cholesterol	16.8±7.0*	29.8±17.3
HDL-cholesterol	44.4±14.7*	57.6±17.5
Serum-apoB	79.0±22.1	95.2±29.2
Serum-apoC Ⅱ	3.3±1.3*	4.4±1.7
Serum-apoC Ⅲ	12.1±3.5	10.0±3.7
Serum-apoE	4.4±1.3	3.9±1.1

Sd-LDL-cholesterol: Small dense LDL-cholesterol
*p < 0.05; **p < 0.01 vs. controls.

Table 3 HDL subfractions in HD patients.

(mg/dl)	HD (n=44) pre HD	post HD	controls (n=10)
HDL-cholesterol	44.4±14.7*/##	53.9±15.8	57.6±17.5
HDL2-cholesterol	33.4±13.9/##	37.1±14.9*	28.0±10.1
HDL3-cholesterol	11.0±4.2**/##	16.6±5.8	29.6±9.2
Serum-apo A Ⅰ	115.4±24.5**/##	145.3±27.7*	167.0±31.3
HDL2-apo A Ⅰ	79.5±22.1#	89.0±25.6*	73.0±19.2
HDL3-apo A Ⅰ	35.9±15.6**/##	51.6±19.6**	93.8±19.2
Serum-apo A Ⅱ	21.8±4.3**/##	26.0±4.4**	35.0±5.8
HDL2-apo A Ⅱ	9.3±2.9**/##	7.8±2.7	6.6±2.3
HDL3-apo A Ⅱ	12.5±3.6**/##	17.5±4.2**	28.4±6.2

*p < 0.05; **p < 0.01 vs. controls.
#p < 0.05; ## p < 0.01 vs. post HD.

Table 1 Clinical characteristics.

	HD patients (n=11)
Male	9
Female	2
DM	5
non DM	6
Age (years)	64.6±15.5
HD duration (months)	52.2±47.2

Table 2. The effects of pitavastatin treatment on serum lipids

(mg/dL)	Baseline	1 Month	3 Months
TC	178.0±31.6	147.9±30.8**	153.1±31.1*
TG	177.3±119.1	140.1±134.1	139.5 ±93.8
LDL-C	99.6±23.7	76.0±17.5**	77.1±20.6*
HDL-C	40.3±13.6	41.9±10.2	46.9±14.2

* $P < 0.05$ compared with baseline.
** $P < 0.01$ compared with baseline.
TC : Total-cholesterol; TG : Triglyceride;
LDL-C : LDL-cholesterol; HDL-C : HDL-cholesterol

Table 3. The effects of pitavastatin treatment on small dense LDL-C, RLP-C and LDL-TG

(mg/dL)	Baseline	1 Month	3 Months
Small dense LDL-C	22.8±13.0	17.3±11.7**	17.5±10.3
RLP-C	8.4±6.5	5.3±3.9**	5.7±3.0*
LDL-TG	19.3±6.9	17.0±4.8	17.6±5.6

** $P < 0.01$ compared with baseline.
* $P < 0.05$ compared with baseline.
RLP-C; remnant-like particle cholesterol

Table 4. The effects of pitavastatin treatment on apolipoproteins

(mg/dL)	Baseline	1 Month	3 Months
ApoB	101.0±23.8	81.1±20.2**	73.5±18.6**
ApoC Ⅱ	4.2±1.8	3.5±1.6**	3.7±1.9
ApoC Ⅲ	13.5±5.4	11.0±4.5**	11.7±4.7
ApoE	4.8±1.3	4.1±1.1**	4.5±1.3

** $P < 0.01$ compared with baseline.

Table 5. The effects of pitavastatin treatment on serum HDL subfractions (pre HD)

(mg/dL)	Baseline	1 Month	3 Months
HDL-C	40.3±13.6	41.9±10.2	46.9±14.2
HDL2-C	27.9±9.9	30.8±7.6*	33.0±10.4
HDL3-C	12.4±4.9	11.0±3.5*	13.9±5.0

* $P < 0.05$ compared with baseline.

Table 6. The effects of pitavastatin treatment on serum HDL subfractions (post HD)

(mg/dL)	Baseline	1 Month	3 Months
HDL-C	54.1±16.8	53.6±12.0	59.9±15.5*
HDL2-C	35.5±10.5	37.9±8.2	38.5±10.0
HDL3-C	18.6±10.0	15.7±6.1	21.4±9.3

* $P < 0.05$ compared with baseline.

Table 7. The effects of pitavastatin treatment on serum-apo A Ⅰ, HDL2-apo A Ⅰ, HDL3-apoA Ⅰ (pre HD)

(mg/dL)	Baseline	1 Month	3 Months
Serum-apoA Ⅰ	138.2±26.0	134.1±21.7	133.0±27.6
HDL2-apoA Ⅰ	81.9±14.8	90.1±12.1*	81.8±17.6
HDL3-apoA Ⅰ	46.0±14.7	35.3±12.2**	45.6±13.8

* $P < 0.05$ compared with baseline.
** $P < 0.01$ compared with baseline.

Table 8. The effects of pitavastatin treatment on serum-apo A Ⅱ, HDL2-apo A Ⅱ, HDL3-apoA Ⅱ (pre HD)

(mg/dL)	Baseline	1 Month	3 Months
Serum-apoA Ⅱ	22.3±2.9	22.9±3.2	25.3±5.3
HDL2-apoA Ⅱ	9.4±3.5	8.9±3.2	8.8±1.4
HDL3-apoA Ⅱ	15.4±5.3	14.9±3.6	15.9±4.5

Results

Protocol 1

1.LDL-C was significantly lower in the HD patients than in the healthy controls.
2.Sd-LDL-C was significantly lower in the HD patients than in the healthy controls.
3.HDL-C was significantly lower in the HD patients than in the controls.
4.HDL-C was significantly lower before HD than after HD .
5.HDL-apoA Ⅰ was significantly lower in the HD patients than in the controls.
6.HDL-apoA Ⅱ was significantly lower in the HD patients than in the controls.
7.HDL-3-C was significantly lower in the HD patients than in the controls.
8.HDL3-apoA Ⅰ was significantly lower in the HD patients than in the controls.
9.HDL3-apoA Ⅱ was significantly lower in the HD patients than in the controls.

Results

Protocol 2

1.LDL-C significantly decreased during pitavastatin treatment.
2.Sd-LDL-C significantly decreased during pitavastatin treatment.
3.RLP-C significantly decreased during pitavastatin treatment.
4.Serum apo B significantly decreased during pitavastatin treatment.
5. HDL2-C significantly increased during pitavastatin treatment.
6. HDL3-C significantly decreased during pitavastatin treatment.
7. HDL2-apo A Ⅰ significantly increased during pitavastatin treatment.
8. HDL3-apo A Ⅰ significantly decreased during pitavastatin treatment.

Discussion (1)

HDL possess anti-atherogenic properties, including a cellular cholesterol efflux capacity and anti-oxidative and anti-inflammatory activities [1]. Serum amyloid A (SAA) is a 12-kDa acute-phase protein [2] which is transported mainly by HDL particles in the blood [1]. A replacement of apoA Ⅰ in HDL particles by SAA plays a role in the reduction of HDL apoA Ⅰ levels in inflammatory states [3] and attenuates the anti-atherogenic activity of HDL [1]. Dialysis patients have higher concentrations of SAA and other inflammatory and proatherogenic substances [4], and this may impair the anti-atherogenic function of HDL.
HDL is typically separated into two major subspecies, HDL2 (*d* 1.063-1.125 g/ml) and HDL3 (*d* 1.125-1.21 g/ml) [5]. HDL3 is small and dense HDL, a lipoprotein with more anti-atherogenic properties than large buoyant HDL (HDL2). Thus, the replacement of apoA Ⅰ by SAA in HDL3 particles presumably results in a more serious impairment of anti-aterogenic function of HDL.

Discussion (2)

Chapman and colleagues proposed that the anti-atherogenic properties are more powerful in small dense HDL (HDL3) particles than in large buoyant HDL (HDL2) particles [1]. SAA can replace apoA Ⅰ in HDL upon induction of the acute phase [6]. HD patients had substantitally low apoA Ⅰ and high SAA levels, which suggests that the replacement of apoA Ⅰ by SAA was stimulated in the HDL particles. We reported that ezetimibe treatment substantially decreased HDL-SAA in HD patients [7]. Statin have been suggested to suppress inflammation and monocyte recruitment [8]. Kimura et al. reported that the results of multivariate analysis identified the amount change of HDL-C associated with increased eGFR during pitavastatin treatment [9]. Nakamura et al. reported that pitavastatin treatment reduced urinary albumin in patients with early diabetic nephropathy, with might be attributable to the antioxidant effects of pitavastatin [10]. In rats, pitavastatin showed a renal protective effect via antioxidant actions, independent of the lipid lowering effects [11]. Pitavastatin may have pleiotropic effects. HDL prevent the oxidized HDL-induced epidermal growth factor receptor activation [12].

Discussion (3)

We have reported that atorvastatin reduced LDL-C, sensitive C-reactive protein (HS-CRP), apoB, apo C Ⅱ and apoE [13].
In this study pitavastatin reduced proinflammatory lipoproteins such as LDL and RLP-C. Pitavastatin may induced inhibition of the absorption of proinflammatory oxidized-cholosterol . Pitavastatin treatment may decreased HDL3-SAA in association with the increased HDL2-apoA Ⅰ, and reduced HDL3-apoA Ⅰ. We speculate that pitavastatin increases apoA Ⅰ on HDL2 particles. The attenuation of HDL2-C by pitavastatin treatment would ameriorate dysfunctions of HDL and restore the anti-atherogenic property of HDL in HD patients.
This study has limitations. The number of the subjects is small and the duration is short. Several studies failed to prevent cardiovascular disease despite their reported favorable biochemical effects on circulating levels of lipids. Study of Heart and Renal Protection (SHARP) provide evidence about the efficacy and safety of lowering LDL cholesterol with the combination of ezetimibe and simvastatin among a wide range of patients with chronic kidney disease [14].

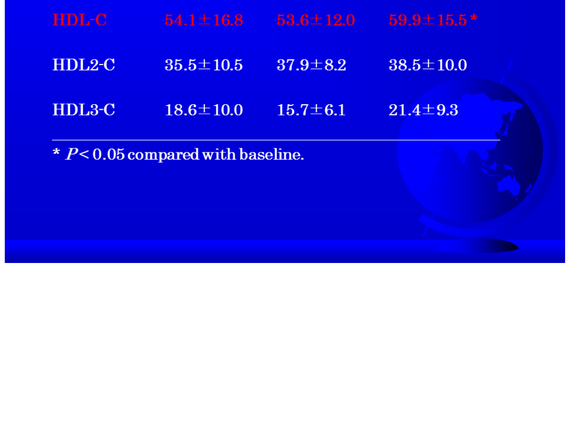

Figure 1. Kontush A and Chapman MJ: Pharmacol Rev 2006; 58:342-374.

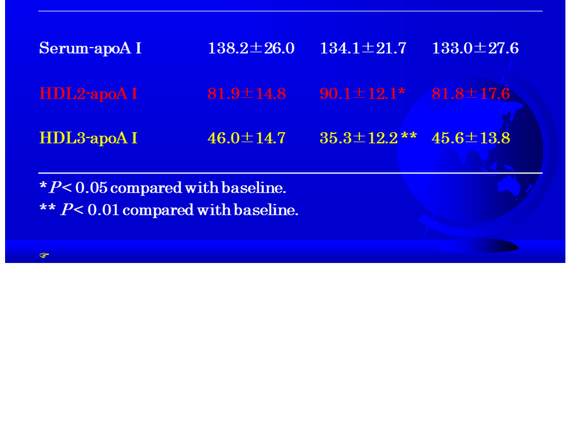

Figure 2.

Conclusion

1. Dyslipidemia in chronic HD patients means the abnormality of the quality and quantity in HDL-C.

2. Pitavastatin increased HDL2-C and HDL2-apo A Ⅰ levels in chronic HD patients.

These data suggest that pitavastatin may restore the anti-atherogenic function of HDL particles in chronic HD patients.

COI: none

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DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM THE NETHERLANDS MAY 31 JUNE 3 2014