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Psoriasis decreases the anti-oxidation and anti-inflammation properties of high-density lipoprotein

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ABSTRACT

Psoriasis is a chronic inflammatory skin disease, which has been linked to dyslipidemia with potential functional impairment of lipoproteins. This cross-sectional study was designed to characterize the biological activities of plasma lipoproteins in 25 patients with psoriasis and 25 age- and sex-matched healthy controls.
In the present study, we found that plasma levels of high-density lipoprotein (HDL) cholesterol were decreased in the psoriasis group compared to healthy controls. The malondialdehyde (MDA) content in plasma, in HDL3 and in low-density lipoprotein (LDL) were increased. However, the activity of plasma paraoxonase-1 (PON-1) decreased in psoriasis and negatively correlated with the psoriasis area and severity index (PASI). Moreover, plasma levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were increased in psoriasis and positively correlated with the PASI. High-sensitivity C-reactive protein (hs-CRP) was increased in psoriasis, but did not reach significance when correlated with PASI. In vitro tests displayed that the functionalities of HDL3 isolated from psoriatic patients significantly decreased, which were assessed in four independent ways, namely (1) protection against LDL oxidation, (2) inhibition of tumor necrosis factor-α (TNF-α) induced monocyte adherence to endothelial cells, (3) prevention of oxidized low density lipoprotein (ox-LDL) induced monocyte migration, and (4) protection of endothelial cells from TNF-α induced apoptosis. Further, pro-oxidative and pro-inflammatory properties of LDL isolated from psoriatic patients were increased. In conclusion, the biological activities of psoriatic lipoproteins are impaired in both HDL and LDL, which may provide a link between psoriasis and cardiovascular disease.

1. Introduction

Psoriasis is a chronic and recurrent inflammatory immune disorder, which is involved in not only skin but also other tissues, such as the cardiovascular system [1,2]. The psoriasis-associated inflammation has a low-grade inflammatory status with elevated C-reactive protein and is noted in both local lesions and entire body tissues. Moreover, psoriasis is associated with a higher incidence of myocardial infarction and cardiovascular mortality [3,4]. Psoriatic patients frequently have traditional risk factors for cardiovascular disease, including dyslipidemia, hypertension, and obesity [5,6]. Psoriasis patients with dyslipidemia have higher values of low-density lipoprotein (LDL) cholesterol and lower concentrations of high-density lipoprotein (HDL) cholesterol [7].

HDL is known to have a strong, inverse correlation with having a risk of atherosclerotic cardiovascular disease. HDL is a complex lipoprotein particle with a broad variety of functions, including potent reverse cholesterol transport, anti-inflammatory capabilities and anti-oxidation properties [8,9]. A recent study clearly shows that inflammation impairs reverse cholesterol transport [10], implying that inflammation destroys HDL functionality. Emerging evidence indicates that psoriasis alters the HDL composition and cholesterol efflux capacity, which could be recovered by anti-psoriatic therapy [11,12]. LDL plays an atherogenic role, and LDL oxidation (ox-LDL) accumulated in psoriatic skin may associate with the immune-inflammatory events and oxidative stress in the pathogenesis of psoriasis [13].

Psoriatic HDL progressively loses normal biological activities in reverse cholesterol transport, but whether psoriatic HDL alters its anti-oxidative or anti-inflammatory activities is unclear. Therefore, we aimed to characterize the biological activities of lipoproteins, especially HDL in psoriasis patients with systemic inflammatory status.

2. Material and methods

2.1. Subjects

The cross-sectional study protocol was approved by the Ethics Committee of Harbin Medical University. All participants obtained written informed consent before collecting the blood samples. The clinical characteristics of study subjects are given in Table 1. Eligible patients met the following criteria: (1) patients were obtained from the outpatient

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Table 1
Clinical characteristics, lipid and apolipoprotein levels of study subjects.

n	Controls	Psoriasis	p
	25	25	–
Age (yr)	43.04 ± 11.15	42.52 ± 14.96	>0.05
Male/Female	12/13	11/14	>0.05
Height (m)	1.70 ± 0.07	1.68 ± 0.08	>0.05
Weight (kg)	60.54 ± 5.55	59.06 ± 5.86	>0.05
BMI (kg/m^2)	20.88 ± 0.78	20.90 ± 0.74	>0.05
PASI	–	15.24 ± 6.87	–
Total cholesterol (mmol/l)	4.28 ± 0.66	4.55 ± 0.44	>0.05
Triglycerides (mmol/l)	1.38 ± 0.22	1.50 ± 0.12	>0.05
HDL-cholesterol(mmol/l)	1.31 ± 0.26	1.15 ± 0.22	<0.05
LDL-cholesterol (mmol/l)	2.37 ± 0.42	2.61 ± 0.40	>0.05
ApoA1 (g/l)	1.19 ± 0.21	1.06 ± 0.23	>0.05
ApoB (g/l)	0.85 ± 0.14	0.92 ± 0.10	>0.05
hs-CRP (mg/l)	1.46 ± 0.67	5.49 ± 3.45	<0.05

Results are given as mean ± standard deviation.
BMI, body mass index; PASI, psoriasis area and severity index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; apoB, apolipoprotein B; apoA1, apolipoprotein A1; hs-CRP, high-sensitivity C-reactive protein.

department of dermatology in the Second Affiliated Hospital of Harbin Medical University; (2) patients were diagnosed with plaque-type psoriasis by at least two dermatologists; (3) patients were at active stage of psoriasis, the patients had presented with one month to ten years of the disease history, and the length of disease relapse was 5 days to 40 days; (4) patients did not use any systemic drugs, such as retinoid and lipid lowering agents, which may influence the blood lipids (some patients were only on small amounts of topical external remedy, without influence in the blood lipids, including calcipotriol (n = 6), tacalcitol (n = 5), dithranol (n = 7), and/or steroids (n = 18) [14]); (5) patients did not have any other systemic disorders, such as angiocardiopathy, diabetes, hypertension, obesity, overweight and so on; and (6) patients did not smoke and drink alcohol. The healthy control participants were recruited from the same geographic region and ethnicity as the psoriasis group. The healthy control participants consisted of outpatients who came to the same hospital for health examinations. These healthy individuals had neither a family history of psoriasis nor any systemic disorders and without using any drugs. The cases and controls were matched for age and gender.

2.2. Plasma analysis

2.2.1. Plasma lipids

Venous blood samples were obtained in the morning after fasting for 12 h. Ethylenediaminetetraacetic acid disodium salt was used as the anticoagulant. Plasma total-cholesterol (TC) and triglyceride (TG) were measured by enzymatic assays methods (Roche, Switzerland), and HDL cholesterol (HDL-C) and LDL-C were quantified by a direct method (SEKISUI, Japan). Apolipoprotein A1 (apoA1) and apolipoprotein B (apoB) were determined by immunoturbidimetry (Roche, Switzerland). And high-sensitivity C-reactive protein (hs-CRP) was detected by immunoturbidimetry (Orion Diagnostica Oy, Finland). All of these were tested with a chemical autoanalyzer (Roche 7600, Switzerland).

2.2.2. Measurement of plasma oxidized factors

Plasma levels of malondialdehyde (MDA), a marker of oxidative stress, were determined by a spectrophotometric measurement of thiobarbituric acid-reactive substances (TBARS) according to the manufacturer's instructions (Nanjing Jiancheng Biochemistry, China). The activity of paraoxonase-1 (PON-1), an anti-oxidant and anti-inflammation enzyme associated with HDL, was measured by adding plasma to 1 ml of Tris–HCl buffer (100 mM, pH 8.0) containing 1 mM $CaCl_2$ and 1 mM of phenylacetate (Sigma) as described previously [15]. The rate of phenyl acetate hydrolysis was determined by spectrophotometrically (Uvikon 930 spectrophotometer, Kontron) at 270 nm. PON-1 activity was expressed in international units (U) per milliliter of plasma. When measuring PON-1 activity in lipoproteins, the activity was expressed in international U per gram of protein.

2.2.3. Measurement of plasma inflammatory factors

Plasma concentrations of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were determined by ELISA kits (Blue gene, Shanghai, China) according to the manufacturer's instructions.

2.3. Isolation of HDL and LDL

The fasted plasma lipoproteins were fractionated by ultracentrifugation at 40,000 rpm in a Beckman Optima LE-80 K into LDL (density = 1.006–1.063 g/ml) and HDL3 (density = 1.125–1.21 g/ml) as previously described [16,17]. After centrifugation, the fractions were dialyzed in PBS at 4 °C, and immediately used for experiments or stored at −80 °C.

2.4. Measuring anti-oxidant properties of HDL

LDL (100 μg protein/ml) was incubated with freshly prepared $CuSO_4$ (10 μmol/l) in the presence or absence of the isolated HDL3 (200 μg protein/ml). After incubation at 37 °C for 2 h, the extent of LDL oxidation was assessed by measuring the TBARS formation [18] via a spectrophotometric method according to the manufacturer's instructions (Nanjing Jiancheng Biochemistry, China).

2.5. Measuring anti-inflammatory properties of HDL

2.5.1. Endothelial cell - monocyte adhesion assay

Monocyte adhesion assays were performed under static conditions, as previously described [19], with minor modifications. Human umbilical vein endothelial cells (HUVECs) were grown to confluence in 96-well plates and pretreated with or without HDL3 (100 μg protein/ml) for 12 hours before stimulation with TNF-α (20 ng/ml) (PEPROTECH USA) or LDL (100 μg protein/ml) for 6 hours. THP-1 cells were labeled with a fluorescent dye, 2', 7'-bis (2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM), by incubation with 10 μmol/L BCECF-AM at 37 °C for 1 hour in the RPMI-1640 medium and were subsequently washed with EBM-2. Confluent HUVECs in 96-well plates were washed 3 times, and labeled THP-1 cells (1×10^4 cells per 100 μL) were added to each well of HUVECs. THP-1 cells were allowed to adhere to HUVECs by incubation at 37 °C for 60 minutes, and unbound THP-1 cells were removed by washing (3 times, 5 minutes each). THP-1 cells bound to HUVECs were counted under the fluorescent microscope. The number of adherent leukocytes was determined by counting 4 fields per × 100 high-power-field well using fluorescent microscopy (Nikon, Japan) and photographed. Four randomly chosen high-power fields were counted per well. Experiments were performed in duplicate or triplicate and were repeated at least 3 times. The person counting the adherent monocyte was unaware of the treatment.

2.5.2. Monocyte migration assay

Conditioned mediums were from ox-LDL-treated (100 μg protein/ml, Beijing Union-biology Co. Ltd) HUVECs for 12 h. Simultaneously, the THP-1 cells were pretreated with HDL3 (100 μg protein/ml) for 12 hours. Then, the conditioned mediums were placed in the lower compartment of a transwell chamber to induce cell migration, and THP-1 cells with HDL3 were added into upper compartment (8 μm BD Falcon). Eight hours later, the upper cells were removed, and the lower cells were stained with BCECF-AM for 1 h. The results of the migration were analyzed by counting the cells in 4 random fields under a fluorescence microscope [20].