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ORIGINAL ARTICLE

Haptoglobin from psoriatic patients exhibits decreased activity in binding haemoglobin and inhibiting lecithin-cholesterol acyltransferase activity

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Keywords
apolipoprotein A-I, haptoglobin, haemoglobin, lecithin-cholesterol acyltransferase, psoriasis vulgaris

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Abstract

Objective The aim of this work was to assess whether psoriasis is associated with phenotype prevalence and altered activity of haptoglobin (Hpt).

Background Hpt is a plasma acute-phase glycoprotein, displaying in humans three phenotypes. Phenotype prevalence or structure modification of Hpt was associated with several diseases. The Hpt main function is to bind and carry to the liver free haemoglobin for degradation and iron recycling. Hpt was recently found able to bind the apolipoprotein A-I (ApoA-I), thus impairing its stimulation on the activity of the enzyme lecithin-cholesterol acyl-transferase (LCAT).

Study design Hpt was isolated from patients with psoriasis vulgaris, and its activity in haemoglobin or ApoA-I binding and LCAT inhibition was compared with that of normal protein.

Methods Two affinity chromatography steps, the first using resin-coupled haemoglobin and the second anti-Hpt antibodies, were used to purify Hpt. The protein phenotype was assessed by electrophoresis. Binding experiments were performed by Enzyme-linked immunosorbent assay with stationary haemoglobin or ApoA-I, Hpt in solution and anti-Hpt antibodies for detection of bound Hpt. Standard LCAT assays were carried out in the presence of Hpt purified from patients or healthy subjects.

Results Phenotype prevalence of Hpt in psoriasis was not found. After affinity chromatography by haemoglobin, albumin and ApoA-I were routinely found heavily contaminating only Hpt from normal subjects. Isolated Hpt from patients had lower activity than normal protein in both haemoglobin binding and LCAT inhibition.

Conclusions In psoriasis, Hpt displays some structure modification(s), which might be associated with the protein function in the disease.

Introduction

Haptoglobin (Hpt) is a plasma acute phase α_2-sialoglycoprotein mostly synthesized by the liver.[1] Evidence that Hpt is expressed in other tissues like lung, kidney, skin and heart is growing up.[2] Its production is regulated by cytokines (interleukin-6, interleukin-1, tumour necrosis factor-α, etc.) and glucocorticoids.[3] Although Hpt is found in the serum of all mammals, only in humans it is characterized by molecular heterogeneity depending on genetic polymorphism and represented by three major phenotypes: Hpt 1-1, Hpt 2-2, and Hpt 1-2.[4] Hpt phenotype prevalence

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appears to be associated to immune response, mainly to autoimmune and inflammatory disorders.[5] Hpt levels markedly increase during the acute-phase response of inflammation and in neoplastic diseases, whereas they decrease in haemolytic and hepatocellular diseases.[1] It has been suggested that Hpt modulates immunological and inflammatory responses.[6–9] The best known biological function of Hpt is the binding of free haemoglobin (Hb) for transport to the liver to prevent iron loss and free radical–mediated renal damage.[10] More recently, Hpt was found able to bind the apolipoprotein A-I (ApoA-I) and impairing *in vitro* the ApoA-I stimulation on the activity of the enzyme lecithin-cholesterol acyltransferase (LCAT).[11] A number of other physiological roles of Hpt were suggested.[12–15] Synthesis of Hpt in the skin was found interfering with LC function by preventing their functional transformation and activating autologous T cells.[16] However, it is not clear whether this is an indirect effect through modulation of antigen-presenting cellular function, or whether Hpt act directly on T cells. Although the importance of extrahepatic Hpt synthesis is not yet understood, local Hpt synthesis may provide tissues with local source of abundant Hpt, which may exhibit differences in structure and function. As a matter of fact, Hpt structure modifications of local Hpt isoforms were found associated with a number of diseases, including vascular disease, rheumatoid arthritis, endometriosis and ovarian cancer.[17–20] In all these disorders, the disease-related Hpt isoforms were detected in plasma, circulating together with normal hepatic isoforms. The aim of this work was to purify Hpt from patients with psoriasis vulgaris and ascertain whether it displays phenotype prevalence in this disease and different Hb or ApoA-I binding property from normal protein.

Materials and methods

Materials

Chemicals of the highest purity, bovine serum albumin (BSA), Hb, cholesterol, cholesteryl linoleate, human Hpt (mixed phenotypes: Hpt 1-1, Hpt 1-2, Hpt 2-2), rabbit anti-human Hpt IgG, goat anti-rabbit horseradish peroxidase (GAR-HRP) IgG, *o*-phenylenediamine and molecular weight markers were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human ApoA-I and rabbit anti-human ApoA-I IgG were from Calbiochem (La Jolla, CA). [1α, 2α-^{3}H] Cholesterol (45 Ci/mmol) was obtained from Perkin-Elmer (Boston, MA, USA). Hi-Trap NHS-activated columns (Amersham Biosciences, Buckinghamshire, UK) and Sil-G plates for thin-layer chromatography (0.25 mm thickness) of Macherey-Nagel (Düren, Germany) were used.

Subjects and clinical evaluation

Patients ($n = 46$) with psoriasis vulgaris and control subjects ($n = 44$) matched for age and cardiovascular risk factors [total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, smoking status, blood pressure] were enrolled after informed consent. The patients had active disease and were not taking anti-inflammatory drugs. No subject had any symptom or laboratory finding of kidney, liver or thyroid dysfunction, infection, diabetes, malignancy, or was taking drugs affecting plasma lipid or lipoprotein levels. The present investigation conforms with the principles outlined in the declaration of Helsinki.

Hpt purification

Venous peripheral blood was drawn into a heparinized tube, and plasma was obtained by centrifugation ($500 \times g$; 15 min; 4°C). Hpt purification was done by affinity chromatography. Hi-Trap NHS-activated resin was used to bind Hb on the stationary phase, according to the manufacturer's instructions. Approximately 10 mg Hb was coupled with 1 mL of resin. The Hb affinity column was equilibrated was 10 volumes of PBS (50 mM Na_2HPO_4/NaH_2PO_4 pH 7.4) and then loaded with 2 mL of plasma at 0.2 mL/min. Plasma samples from the different individuals were separately processed. The column flow through was loaded again for three times to achieve total binding of the Hpt isoforms. The Hpt-loaded resin was extensively washed, to remove unbound material with PBS at 1 mL/min flow rate. When the effluent absorbance at 280 nm was no longer detected, Hpt was eluted, at first with 15 mL of 0.2 M glycine-HCl at pH 3.5, then with 10 mL of 0.2 M glycine-HCl at pH 2.8. Fractions of 0.5 mL were collected into tubes containing 1 M. Tris base in order to adjust fraction pH and analysed for their absorbance at 280 nm (A_{280}) to monitor protein elution. This Hpt preparation was used to detect the protein phenotype but was not suitable for assaying the protein activity owing to the presence of contaminants. In order to isolate Hpt, the material from chromatography with resin-linked Hb was processed by a further step of affinity chromatography, using a small column of anti-Hpt IgG coupled to the stationary phase. Sample loading, column washing and protein elution were done as for affinity chromatography with Hb. The Hpt phenotype was determined as reported elsewhere.[21]

Electrophoresis and densitometry

Electrophoresis in denaturing and reducing condition was carried out on 1.5-mm-thick slab gel, using the

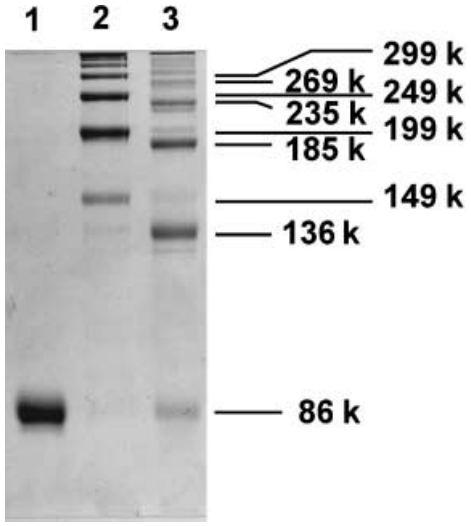

fig. 1 Hpt phenotyping. Hpt, as obtained by affinity chromatography with Hb coupled to stationary phase, was analysed. Aliquots of 40 μg were loaded on 6% polyacrylamide gel, and the electrophoresis was carried out in denaturing but non-reducing conditions. The protein pattern of Hpt 1-1 (*lane 1*), Hpt 1-2 (*lane 2*) and Hpt 2-2 (*lane 3*) was detected by staining with Coomassie R-250. *Right*, the molecular mass of different isoforms is indicated accordingly to previously published data [Cigliano ABB2003].

discontinuous system described by Laemmli but with a separating gel containing 15% or 10% polyacrylamide.[22] Sample containing 3 to 5 μg of protein were boiled in the O'Farrell buffer O, containing sodium dodecylsulphate and β-mercaptoethanol, for 5 min before loading on the gel.[23] Molecular weight markers (BSA, 66 kDa; egg albumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20 kDa; and α-lactalbumin 14 kDa) were used to measure the protein electrophoretic mobility. Protein fractionation was achieved in 1 h at 100 V. Electrophoresis in denaturing and non-reducing condition was done on 6% polyacrylamide gel to separate Hpt isoforms (fig. 1). In particular, purified Hpt samples (3–5 μg) were incubated for 5 min at 37 °C in modified O'Farrell buffer O (i.e. the buffer prepared without β-mercaptoethanol) and then loaded on the gel. Electrophoresis was carried out for 2 h at 100 V. After electrophoresis, the gel was fixed in acetic acid/isopropanol/water (10 : 25 : 65, v/v/v) and stained with Coomassie R-250 (0.05%, w/v, in the fixing solution) for 2 h at room temperature. Destaining was accomplished by repeatedly shaking the gel in 50 volumes of acetic acid/water (10 : 90, v/v) until a transparent background was obtained.

Protein electrophoresis patterns were analysed by densitometry as follows. Digital images of gels were analysed by the Gel-Pro Analyser software (Media Cybernetics, Silver Spring, MA). The band intensities were recorded as peaks on a densitogram. Densitometric signals were arbitrarily expressed as Integrated Optical Density (IOD). On the basis of a calibration using known amounts of standard BSA, it was determined that 1 IOD corresponds to the intensity of 5 ng of Coomassie-stained protein.

Enzyme-linked immunosorbent assay

Purified fractions of Hpt were analysed for their Hpt concentration by enzyme-linked immunosorbent assay (ELISA), using anti-Hpt and GAR-HRP IgGs according to a published procedure.[21] Wells treated, omitting the sample or the primary antibody incubation, were used to evaluate the colour development background or non-specific binding of the secondary antibody. The calibration curve was obtained by assaying the immunoreactivity of 0.1, 0.25, 0.50, 0.75, 1.0, and 2.0 ng of commercial protein standard. The standard concentration was determined by a colorimetric assay using purified IgG for calibration.[24] In experiments of Hpt binding to Hb, microtiter plate wells were coated by incubation with 0.4 μg of Hb in coating buffer [7 mM Na_2CO_3, 17 mM $NaHCO_3$, 1.5 mM NaN_3 (pH 9.6)]. Then, different amounts of Hpt (0.05, 0.1, 0.2 and 0.3 μM) in dilution buffer [DB: 0.2% BSA, 20 mM Tris (pH 7.3), 130 mM NaCl, 0.05% Tween 20] were incubated in the wells for 1.5 h at 37 °C. The binding of Hpt to Hb was detected by anti-Hpt IgG (1 : 5000), GAR-HRP IgG (1 : 10 000) and colour development was monitored at 492 nm as previously described.[11]

Calculation of Hpt molarity

Because both Hpt 1-2 and Hpt 2-2 are oligomeric proteins, each displaying a mixture of isoforms with different number of monomers,[21] the protein molarity was expressed as concentration of monomer [i.e. the structure unit containing one subunit β (40 kDa) and one subunit α (α_1, 8.9 kDa, or α_2, 16 kDa)].[1] The monomer can be considered the functional unit of Hpt because the subunit β harbours the domains involved in binding Hb[25,26] or ApoA-I.[27] Thus, molarity of Hpt 2-2 isoforms, which contain only $\alpha_2\beta$ units assembled in circular molecules, was expressed as $\alpha_2\beta$ molarity, and MW = 56 kDa was used for calculation. Differently from Hpt 2-2 isoforms, those of Hpt 1-2 are linear molecules constituted by differently long sequences of $\alpha_2\beta$ units with $\alpha_1\beta$ units at their ends. Therefore, to calculate for Hpt 1-2 the monomer molarity (i.e. $\alpha_1\beta$ molarity + $\alpha_2\beta$ molarity), the relative amount of $\alpha_2\beta$ and $\alpha_1\beta$ in the isoform mixture isolated from different subjects was determined. In particular, the Hpt 1-2 subunits were fractionated by

electrophoresis in denaturing and reducing conditions, and the intensities of the Coomassie-stained bands were measured by densitometry.[21] The equations [α_1 intensity] /[α_2 intensity] = [$x \times 8900$]/[$y \times 16\,000$] and [average molecular mass of subunit α] = [$x \times 8900$] + [$y \times 16\,000$], where x (relative amount of α_1, expressed in grams) + y (relative amount of α_2, expressed in grams) = 1, were used to calculate the average molecular mass of α. Such a value, which was different from different protein samples, was used to calculate the monomer molarity in Hpt 1-2. In this work, molecular mass values ranging from 48.9 to 56 kDa were assigned to the monomer, depending on isoform population present in any purified Hpt 1-2.

LCAT assay

A pool of plasma samples, treated 0.65% with dextran sulphate (DS; MW = 50 kDa) in 0.2 M $CaCl_2$ to remove very low density lipoprotein, LDL and HDL, was used as source of LCAT (DS-treated plasma). The enzyme activity was measured using a proteoliposome (ApoA-I/lecithin/cholesterol, 1.5 : 200 : 18, molar contribution) as substrate, essentially according to published procedures.[28,29] In detail, 8 μL of 50 mg/mL egg lecithin in ethanol were mixed with 18 μL of 1 mg/mL cholesterol in ethanol, 40 μL of [1,2-^{3}H(N)]-cholesterol (1 μCi/mL) into a glass vial. The solvent was carefully evaporated under nitrogen stream at room temperature and to the dried lipids 170 μL of a suspension medium [85 mM sodium cholate, 150 mM NaCl, 10 mM Tris–HCl (pH 8)] were added. After vigorous whirling (3 min, room temperature), the micelle suspension was incubated (90 min, 37 °C) and repeatedly shaken every 10 min until clear. Then, 90 μL of 1.21 mg/mL ApoA-I were added to the lipid suspension, which was further incubated for 1 h at 37 °C. The resulting proteoliposome suspension was extensively dialysed against Tris-buffered saline solution containing EDTA [TBE: 140 mM NaCl, 1 mM EDTA, 10 mM Tris–HCl (pH 7.3)], at 4 °C, to remove cholate. The resulting volume was adjusted to 285 μL using TBE. The reaction mixture (1 mL final volume) was prepared by putting together 697 μL of TBE containing 5 mM $CaCl_2$, 83 μL of 6% HSA, and 160 μL of proteoliposome suspension (diluted 1 : 20 in TBE) into a screw-capped tube and heating at 38 °C for 30 min. The assay was carried out by addition of 2.5 μL of 2 mM β-mercaptoethanol and 4 μL of DS-treated plasma to 100 μL of reaction mixture, which was rapidly divided into three aliquots of 32 μL and incubated (1 h, 37 °C). Aliquots of Hpt were added, when required, to the reaction mixture before incubation, and the protein molarity was calculated as above described. The reaction was stopped by addition of 130 μL of ethanol to each aliquot. The lipids were extracted in 600 μL of hexane, containing 10 μg/mL cholesterol and 10 μg/mL cholesteryl linoleate. After recovering the organic phase, the aqueous phase was again treated with 500 μL of the extraction solution (twice), and the three extracts were pooled. Hexane was removed under nitrogen stream, and the dried lipids were dissolved in 50 μL of chloroform. Cholesteryl esters were separated from cholesterol by thin layer chromatography, using petroleum ether, diethyl ether, and acetic acid (90 : 30 : 1, v/v/v) as mobile phase. The lipid spots were visualized under iodine vapour and recovered for scintillation analysis. The enzyme activity was measured as nmoles of cholesterol esterified per h per mL of plasma and expressed as units.

Statistics

The Jack-knifing test was used to predict the number of patients or healthy subjects sufficient to significantly assess phenotype prevalence. In detail, Hpt phenotype of patients or healthy subjects was found, seven groups of sorting (pick up number = 10, 15, 20, 25, 30, 35 and 40) were chosen, and 10 drawings per group were done to calculate means and SD values. The SD values were plotted vs. the relevant pick up number to obtain trend curve, equation and r^2 for each phenotype. Equations ($r^2 > 0.99$) were solved for SD = 0, and the subject number resulted to be sufficient for assessing significance.

Samples in the ELISA and LCAT assays were analysed in triplicate. The program 'Graph Pad Prism 3' (GraphPad Software, San Diego, CA) was used to perform regression analysis or *t*-test.

Results

Hpt phenotype prevalence in psoriasis

Hpt was purified from individual plasma of healthy donors, or patients with psoriasis. The protein was positively selected by affinity chromatography, using Hb coupled to stationary phase. The Hpt phenotype was assessed by electrophoresis on 6% polyacrylamide gel (fig. 1). In detail, after electrophoresis, the gel was stained by Coomassie and analysed for the presence of protein bands corresponding to Hpt isoforms, which are present in phenotype 1-1 (86 kDa), 2-2 (149, 199, 249 and 299 kDa), or 1-2 (86, 136, 185, 235 and 269 kDa). As shown in Table 1, the phenotype Hpt 1-2 was prevalent in healthy subjects, according to previous information on European phenotypic distribution.[1] Among the patients, Hpt 1-2 was frequent as Hpt 2-2, and prevalence of either phenotype was not assessed. The number of subjects with Hpt 1-1 was too low to appreciate a possible difference in frequency of this phenotype between patients and controls.

Table 1 Phenotype prevalence of Hpt in psoriasis

Hpt phenotype	Patients ($n = 46$)	Controls ($n = 44$)
1-1	3	2
1-2	22	24
2-2	21	18

Hpt preparation and ApoA-I titration

Hpt, as obtained by affinity chromatography with resin-linked Hb, is often accompanied by small amounts of two proteins (i.e. albumin and ApoA-I).[30] We noted that the contaminant proteins were mainly present in preparations of Hpt from normal subjects, whereas only traces (if any) could be detected by gel electrophoresis and densitometry in Hpt isolated from patients (fig. 2). Owing to the relevance of the interaction of Hpt with ApoA-I in the reverse cholesterol transport, we wondered whether the observed difference in ApoA-I contamination between Hpt samples from patients and those from healthy subjects would depend on different plasma levels of ApoA-I. Thus ApoA-I was titrated in plasma from both patients and controls by ELISA (1.108 ± 0.095 μg/mL, $n = 5$ and 1.12 ± 0.073 μg/mL, $n = 5$, respectively), and no significant difference in ApoA-I concentration between the two groups was found.

Effect of Hpt from patients or controls on LCAT activity

Previous data showed that Hpt, by binding ApoA-I, inhibits the LCAT enzyme by preventing the ApoA-I-dependent stimulation of the catalytic activity.[11] The lower contamination of ApoA-I present in Hpt preparations from psoriatic patients, compared with those from healthy donors, suggests that (some) Hpt isoforms might be associated with the disease and display differences in structure from normal isoforms. To support such a hypothesis, the function of normal and disease-associated Hpt was tested in the LCAT assay. Four separated samples of Hpt (two Hpt 1-2 and two Hpt 2-2) from Hb-affinity chromatography of plasma from patients were processed by affinity chromatography with antihuman Hpt, as described in the method section, in order to obtain Hpt free of contaminants. The protein purity was over 98%, as evaluated by gel electrophoresis (data not shown). A pool of two Hpt 1-2 and two Hpt 2-2 from healthy donors was prepared and used as control. Aliquots of the two different pools were mixed with DS-treated plasma, used as LCAT source, and proteoliposomes containing ApoA-I and labelled cholesterol. The final Hpt concentration in the assay mixture was 0.5 or 2 μM Hpt. After incubation, inhibition of the enzyme activity by normal Hpt was clearly detected at both concentration used, as expected, whereas it was significant but lower than normal when the enzyme assay was carried out with 2 μM Hpt from patients (fig. 3). The difference between the two Hpt pools in inhibiting LCAT suggests that the protein purified from

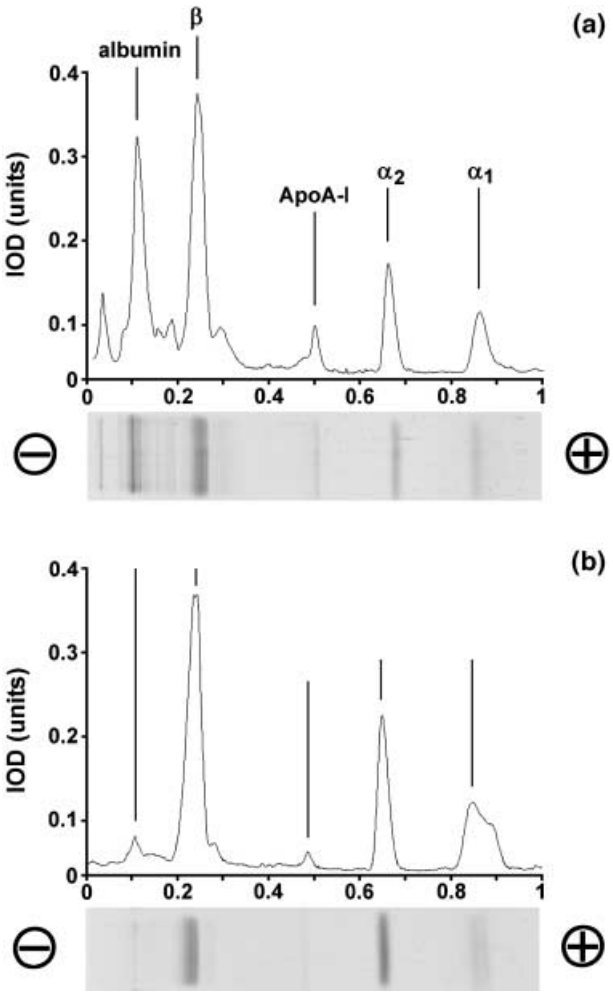

fig. 2 Densitometric analysis of Hpt prepared by Hb affinity chromatography from plasma of patients or healthy subjects. Samples of Hpt, obtained by affinity chromatography with Hb coupled to Sepharose, were processed by electrophoresis on 15% polyacrylamide gel in denaturing and reducing conditions. After staining with Coomassie R-250 and extensive destaining in acetic acid/water (10 : 90, v/v), digital images were taken by a scanner with adapter transparency unit for positive film, at resolution of 600 pixels per inch. The images were analysed by the software Gel-Pro, which converted the band intensities in optical densities. Densitograms represent changes of optical density as function of the electrophoretic migration. Typical densitograms of samples from patient (a) and healthy subject (b). The electrophoretic mobility of albumin, ApoA-I, and the Hpt chains α and β is indicated. Images of protein electrophoretic fractionation used for densitometry (*bottom*). Migration toward cathode is expressed as Rf.

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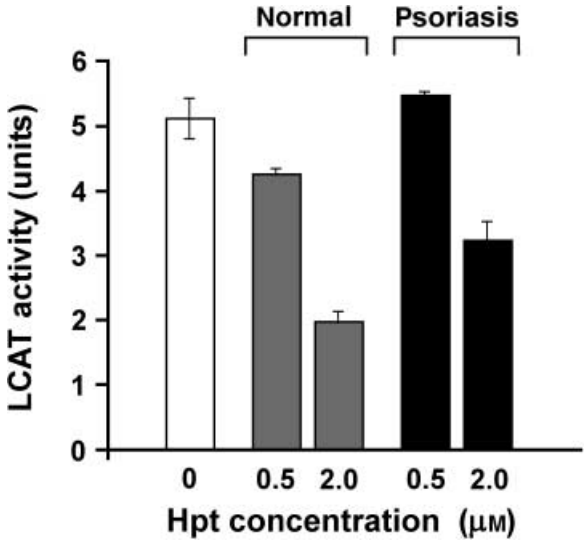

fig. 3 Inhibition of LCAT activity by Hpt from patients or healthy subjects. The LCAT activity was assayed by incubating a pool of DS-treated plasma with a standard reaction mixture, containing a proteoliposome (ApoA-I: lecithin: ^{3}H-cholesterol, 1.5 : 200 : 18 molar ratio) as substrate. The enzyme activity was measured in the presence of Hpt (0.5 or 2 μM), purified from patients or healthy subjects, and pooled. Pools with equimolar amounts of Hpt (two Hpt 1-2 samples and two Hpt 2-2 samples) were used. A control assay was performed without Hpt. The LCAT activity was expressed as nanomoles of cholesterol incorporated per hour per ml of plasma. The samples were analysed in triplicate. *Columns*, means; *bars*, SE. The enzyme activity, as measured in the absence of Hpt (*white column*), was significantly different from that in the presence of both 0.5 or 2 μM Hpt of healthy subjects and 0.5 μM Hpt from patients ($P < 0.001$). Differences between 0.5 and 2 μM samples from the same pool and between the two pools at either concentration were also significant ($P < 0.001$).

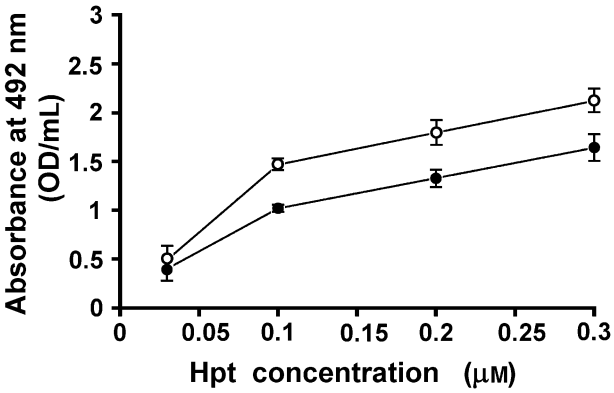

fig. 4 Binding of Hpt from patients or healthy subjects to Hb. Pools with equimolar amounts of purified Hpt (two Hpt 1-2 samples and two Hpt 2-2 samples) were prepared. Wells of a microtiter plate were coated with Hb, and incubated with different amounts of pooled Hpt from patients (*full circles*) or healthy subjects (*open circles*). Anti-Hpt rabbit were used to bind the formed Hpt–Hb complexes. Following incubation with GAR-HRP and *o*-phenylenediamine in the presence of H_2O_2, the complexes were detected by measuring the solution absorbance at 492 nm. The samples were analysed in triplicate. *Points*, means; *bars*, SE. Values at 0.1, 0.2 and 0.3 μM Hpt are significantly different ($P < 0.001$).

patients has not the same structure of normal protein. This difference might depend on lower ability of Hpt from patients to limit ApoA-I stimulation of stimulating LCAT activity.

Binding of Hpt to ApoA-I or Hb

Differences in Hpt preparation by affinity chromatography with Hb might underlie different affinity of Hpt for ApoA-I or Hb. Therefore, Hpt was prepared, free of contaminants, from patients with psoriasis ($n = 4$; two with Hpt 1-2 and two with Hpt 2-2) and tested for its activity of binding ApoA-I or Hb. Such an activity was compared with that of Hpt isolated from healthy donors ($n = 4$; two with Hpt 1-2 and two with Hpt 2-2). The wells of a microtiter plate were coated with ApoA-I or Hb and incubated with different amounts of Hpt (0.03, 0.1, 0.2 and 0.3 μM). Unbound Hpt was removed by extensive washing, and the complexes of Hpt with ApoA-I or Hb were targeted by rabbit anti-Hpt antibodies, which were on turn detected by peroxidase-linked goat anti-rabbit antibodies with *o*-phenylenediamine and hydrogen peroxide. No significant difference in ApoA-I binding between Hpt from patients and that from controls, at any used concentration in the assay, was detected. Conversely, the results indicate Hpt from normal subjects bound Hb more efficiently than that from patients (fig. 4). In particular, significant differences were found for samples with 0.1, 0.2 or 0.3 μM Hpt ($P < 0.001$).

Discussion

The Hpt polymorphism in several diseases was reviewed, but information on phenotype prevalence in psoriasis was not provided.[1,31–33] In this study, data on decreased frequency of the Hpt 1-1 phenotype in psoriasis are reported. Prevalence of Hpt 2-2 isoforms was expected in psoriasis because they stimulate angiogenesis, a well-known phenomenon associated with the disease, more than isoforms of the other two phenotypes.[14] Prevalence of Hpt 2-2 was found in several diseases, and this phenotype was recognized as risk factor for cardiovascular diseases.[1,31–33] Biochemical mechanisms underlying the atherogenic properties of Hpt were suggested,[34–36] but molecular evidence on why Hpt 2-2 associates to higher risk than Hpt 1-1 or Hpt 1-2 is lacking to date. Psoriasis has been associated with abnormal plasma lipid metabolism and with high frequency of cardiovascular events.[37–44]

This prevalence of cardiovascular events seems to be related to the severity of psoriasis, considering that it occurs much more frequently in patients presenting large areas of the body affected with psoriasis lesions.[45] However, we did not found prevalence of Hpt 2-2 in psoriasis. Therefore, Hpt phenotype prevalence does not represent a link between psoriasis and cardiovascular diseases. Abnormal plasma lipid metabolism, in the onset and progression of cardiovascular diseases, mostly depends on poor reverse cholesterol transport. In this process, ApoA-I stimulates cholesterol efflux from peripheral cells and activates LCAT to synthesize cholesteryl esters, which are then embedded into HDL for transport to liver. Hpt binds ApoA-I, thus impairing the apolipoprotein stimulation on the enzyme activity.[11] Our results show that Hpt from patients with psoriasis inhibits cholesterol esterification less than normal Hpt. This means that some disease-associated difference in Hpt structure, not dependent on the protein phenotype, might play a role in the regulation of the LCAT activity. We cannot exclude that some difference in LCAT activity between patients and controls might be observed *in vivo*, depending on disease-associated function alteration of ApoA-I, LCAT or lipids. Oxidative stress, known to occur in inflammation, might actually be responsible of structure and function modifications of all these molecules. However, the results obtained in our experiment of LCAT stimulation *in vitro* clearly indicate that at least the Hpt alteration affects the enzyme activity. In fact, in this experiment, Hpt was the only variable between patients and controls.

Here we provide evidence that, in psoriasis, plasma contains Hpt isoforms different from those circulating in normal conditions. In particular, differences in binding of albumin, ApoA-I and Hb were observed. Such function differences seem not to depend on the Hpt phenotype because both Hpt 1-2 and Hpt 2-2 isoforms from patients displayed lower binding activity. Poor information is available on the interaction of Hpt with albumin, and the significance of such an interaction is not clear.[46] It is possible that albumin binds Hpt to prevent degradation or aggregate formation of this protein. It cannot be excluded that albumin does not bind Hpt but interacts with ApoA-I. In this case, the affinity chromatography would capture ApoA-I–bound albumin, and a role for albumin could be envisaged according to previously published data, which showed albumin requirement for optimal ApoA-I stimulation on the LCAT activity.[47] As a matter of fact, Hpt from patients displayed lower activity in the LCAT assay as abovementioned. This means that, in the assay, Hpt binding to ApoA-I was limited, as expected if albumin would be required for stabilizing the Hpt-ApoA-I complex. Therefore, albumin, as binding normal Hpt better than that present in the patient plasma, would be responsible of the different amounts of ApoA-I selected by affinity chromatography of samples from patients or controls. However, the finding of higher amounts of albumin in Hpt preparations from healthy subjects than in those from patients suggests that Hpt with structure changes is present in psoriasis. Further evidence on psoriasis-associated isoforms of Hpt was gained by Hb or ApoA-I binding experiments. Our data indicate that Hpt from patients binds Hb less efficiently than Hpt from controls, whereas no difference in binding purified ApoA-I was observed. The Hpt-linked glycan chains were shown to be involved in the formation of the Hpt-Hb complex,[48] and changes in these glycan structures were previously found in diseases associated to inflammatory conditions.[49–51] It is therefore conceivable that Hpt, in psoriasis, might expose glycans altered in their structure and function in Hb binding. Further glycosylation analysis of Hpt from psoriasis is required to support this hypothesis. Hpt glycan or amino acid domains involved in the Hb binding do not participate in the ApoA-I binding.[11,52] It remains to be discussed why disease-associated Hpt isoforms would bind ApoA-I in the LCAT assay less than normal Hpt, whereas no difference between patients and controls was observed by ELISA in this binding. A possible explanation is that albumin might contribute to stabilize the interaction between Hpt and ApoA-I in the enzyme assay, whereas its effect might be poor (if any) in ELISA conditions.

In conclusion, we provide here evidence that Hpt, in psoriasis, displays structure modification(s) that might impair the protein function in Hb binding and LCAT regulation. Structure analysis of Hpt isoforms in psoriasis and normal conditions are in progress to validate our hypothesis.

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