CONCISE REPORT

The Degradation of Platelet-Activating Factor in the Plasma of a Patient with Familial High Density Lipoprotein Deficiency (Tangier Disease)

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Platelet Activating Factor (PAF) (1-O-alkyl-2-acetyl sn-glycerol 3-phosphocholine) has been characterized by its ability to aggregate platelets at low concentrations and its profound hypotensive effects. There is evidence that the rate of catabolism of this compound in the plasma regulates its concentration. In humans, we and others have shown that a PAF acetylhydrolase is associated with low density lipoprotein (LDL). The LDL particle in the plasma of patients with Tangier disease is quite different from normal as its lipid core appears to be enriched with triacylglycerol. Thus, we have studied the potential of this abnormal lipoprotein to degrade PAF. The assay for PAF acetylhydrolase was based on the release of $^3$H from PAF that was labelled in the acetate moiety of the sn-2 position. Tangier disease plasma had approximately 3.3-fold higher PAF acetylhydrolase activity (208 ± 9 nmol/min/mL) than controls (63 ± 18 nmol/min/mL). This increase was brought about by an increase in the Vmax (400 ± 40, Tangier disease; 54 ± 5, controls) and Km for PAF (120 ± 20 μmol/L, Tangier disease; 28 ± 4 μmol/L, controls). The activity appears to be a specific acetylhydrolase rather than a phospholipase A2 as preincubation of the substrate with 0 to 100 μmol/L phosphatidylcholine did not affect the amount of $^3$H acetate released. The role of PAF, and its degradation by LDL-bound PAF acetylhydrolase in the phenotypic expression of this patient with Tangier disease, is not known. However, this is the first patient so far described who has an increased ability to degrade PAF in the plasma.

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TANGIER DISEASE is a rare autosomal recessive disorder characterized by the virtual absence of high density lipoprotein (HDL) in the patients plasma and an accumulation of cholesteryl esters in a number of peripheral tissues. The exact genetic defect in this disorder has not yet been fully elucidated. However, it has been proposed that a defect in the posttranslational modification of apolipoprotein A-I in the plasma of patients with Tangier disease produces an abnormal particle that is rapidly catabolized. In addition, we have recently proposed that HDL may be removed from the plasma compartment by increased cellular metabolism. We have initiated a series of experiments to test the hypothesis that the plasma modification of HDL lipid is abnormal in patients with Tangier disease. Specifically, we are interested in the role of phospholipase activities in regulating the phospholipid pool of the HDL particles. However, little is known of the role of these enzymes in regulating the metabolism of the plasma lipoproteins. Increased breakdown of low density lipoprotein (LDL) phospholipids appears to be involved in the biological modification of this lipoprotein which results in an increased rate of catabolism. Thus, we have become interested in phospholipase activities in regulating HDL metabolism in Tangier Disease.

Farr et al have reported the presence of a phospholipase activity that is associated with the LDL class of lipoproteins in human plasma. However, this activity appears to be specific for phosphatidylcholine that contains an acetate moiety in the sn-2 position. It appears that this phospholipase is specific for a group of biologically active phospholipids known collectively as platelet-activating factor (PAF) (1-O-alkyl-2-acetyl sn-glycerol 3-phosphocholine). PAF has been shown to aggregate platelets at low concentrations and to exhibit profound hypotensive effects. As part of our studies on the role of phospholipase activities in lipoprotein metabolism, we have investigated the activity of the PAF acetylhydrolase in a patient with Tangier disease.

We report here that, in Tangier disease, there is a 3.3-fold increase in PAF acetylhydrolase activity that is due to an increase in Km and Vmax of this enzyme for its PAF substrate. This is the first patient so far described with abnormal PAF metabolism.

MATERIALS AND METHODS

Blood samples. As Tangier disease is an extremely rare disorder with only 34 cases reported so far, only one patient with the homozygous form of this disorder was available for this study. We have reported on his clinical, morphologic, hematologic, and biochemical presentation. Controls for this study were healthy male volunteers. Blood was drawn by venipuncture into EDTA tubes and plasma samples were stored at 4 °C until analyzed (less than 4 days).

Column chromatography. Two milliliter aliquots of plasma were incubated for 16 hours at 4 °C in the presence of 10 μCi [7-3H] cholesterol (23.7 Ci/mmol). The samples were then applied to a Biogel A5.0m chromatographic column (90 × 1.5 cm) and eluted at 8 mL/h with 0.15 mol/L NaCl containing 0.15 mol/L Tris/HCl, pH 7.4, 0.01% EDTA, and 0.3% NaNO. Eluant from the column was collected in 2 mL fractions. As 90% of the radiolabel was recovered in the lipoprotein classes, the assay of each column fraction for $^3$H permitted us to establish the elution profile of the different plasma lipoproteins. Moreover, as sufficient time was permitted for the complete equilibration of the radiolabel prior to the chromatography, the amount of label recovered represented the relative amount of cholesterol mass present in each lipoprotein class. The resulting peaks were identified by calibrating the column with authentic human 125I-VLDL, LDL, and HDL prior to each experiment.

Preparation of 1-O-alkyl-2-[acetetyl-3H] sn-glycerol 3-phosphocholine (facetetyl-3H) PAF. Ten milligrams lyso-PAF, 3 mg
dimethylaminopyridine, and 25 mCi [1-^H] acetic anhydride were dissolved in 1 mL methylene chloride. After 12 hours of constant stirring at 30 °C, the lipids were extracted and dried down under nitrogen. The lipid was taken up in chloroform: methanol (4:1). The [acetyl-^H] PAF was purified by thin-layer chromatography on silica G plates developed in chloroform: methanol: acetic acid: water (50:25:8:4, by vol). The radioactive peak corresponding to authentic PAF was scraped off the plate and eluted. The product was characterized by the ability of phospholipase A2 (Naja mozambique) to release the ^H acetate. In addition, the [acetyl-^H] PAF was also capable of aggregating human platelet-rich plasma at 2 × 10^{-7} mol/L.

Assay of PAF acetylhydrolase. This activity was measured in the plasma and in column eluant fractions by a method similar to that described by Blank et al. The assay mixture contained 50 μmol/L [acetyl-^H] PAF (2 to 5 nCi/nmol), 0.15 mol/L phosphate buffered saline, pH 7.4, and 100 μL column fractions or 5 μL plasma (final volume = 0.4 mL). The mixtures were incubated for 10 minutes at 37 °C and the lipids extracted by the method of Folch et al. The amount of ^H recovered in the aqueous phase was determined by liquid scintillation counting. The assays were linear for up to 15 minutes and 10 μL plasma.

RESULTS AND DISCUSSION

We have established a normal range for PAF acetylhydrolase in healthy volunteers of 63 ± 18 nmol/min/mL plasma (n = 19). However, four independent measurements of this activity in different plasma samples from a patient with Tangier disease gave a value of 208 ± 9 nmol/min/mL plasma. Farr et al have reported that this enzyme is associated with the LDL in human plasma. Thus, we have carried out experiments to study the relationship between the plasma lipoproteins and PAF inactivation in this patient.

Figure 1 shows the fractionation of normal and Tangier disease plasma by Biogel A5.0m column chromatography. Very low density lipoprotein (VLDL), LDL, and high density lipoprotein (HDL) from normal plasma were eluted at fractions 28-32, 35-54, and 54-70, respectively. The relative absence of label recovered in fractions 34-70 in the Tangier disease plasma clearly indicates the absence of the HDL particle in this patient. In addition, the increased label recovered in VLDL and in a particle that appears to be between VLDL and LDL in size (fractions 30-45) are also characteristic of the abnormal lipoprotein profile in this disease.

When PAF acetylhydrolase was assayed in the column fractions we found only one peak of activity for both normal and Tangier plasma (Fig 2). This was found in fractions 35-55 that contained the LDL. This confirms the observation of other workers who have suggested that this activity is associated with this lipoprotein. Our data also demonstrates that the increased PAF acetylhydrolase activity in the plasma of Tangier disease all associated with LDL (Fig 2). The activity in both normals and Tangier disease appears specific for PAF as the addition of 0 to 100 μmol/L egg yolk...
the individual plasma samples.

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density lipoprotein subfractions and Tangier disease following the
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disease, the increased degradation of PAF is not due to
upon the rate of release of 3H-acetate. Thus, in Tangier
Vmax was determined from the appropriate intercepts for each of
in Tangier
PAF acetylhydrolase
assayed
in triplicate
from a patient with HDL-deficiency (Tangier Disease). Atheroscle-
metabolism of cholesterol and lipoproteins in skin fibroblast taken
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plasma to degrade PAF and have described the first patient with an abnormally high PAF acetylhydrolase activity. The studies of Blank et al in rats have indicated that increased PAF acetylhydrolase activity is associated with hypertension. This suggests that PAF plays a role in the regulation of blood pressure in these animals. However, despite an increased capacity to degrade PAF in Tangier disease, our patient is normotensive. From this we conclude that, at least in Tangier disease, PAF does not play a systemic role in
the regulation of blood pressure.

The role of lipoproteins in regulating PAF concentrations in the vascular and interstitial compartments is not known. It is possible that they are able to adsorb excess PAF that does not bind to the target cell receptors. This is supported by the observation that monomeric PAF is rapidly and nonspecifically transferred between membrane bilayers in vitro. Thus, as the acetylhydrolase is associated with LDL, this lipoprotein may be pivotal in bringing the unneeded PAF into close proximity with the enzyme that inactivates it.

The relevance of PAF acetylhydrolase in Tangier disease is difficult to assess in the present experiments. Without doubt the activity is abnormally high but we cannot deduce whether or not abnormal PAF metabolism is involved in the phenotypic expression of this disorder. It seems more probable that the increased PAF acetylhydrolase activity is part of a general increase in plasma phospholipase activities and is not directly involved in the genetic defect.

REFERENCES


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