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Blood histamine is associated with coronary artery disease, cardiac events and severity of inflammation and atherosclerosis

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Abstract

Background: Mast cells are prevalent in the shoulder of unstable atheromas; cardiac mast cells secrete proteases capable of activating matrix metalloproteinases. Histamine is essential in the inflammatory cascade of the unstable plaque. Ascorbate depletion has been correlated with histaminemia which has been shown to impair endothelial-dependent vasodilation. This study evaluates whether oxidative stress as measured by isoprostanes ($PGF_{2\alpha}$) coupled with an inflammatory state characterized by histaminemia predisposes patients to acute coronary syndrome (ACS).
Methods: Whole blood histamine, serum vitamin C, and serum $PGF_{2\alpha}$ levels were drawn on 50 patients with ACS as determined by standard diagnostic criteria, 50 patients with stable coronary artery disease (SCAD), and 50 age and sex matched normal controls (C).
Results: Data were analyzed by stepwise discriminant and Spearman's rank correlation coefficient. A significant relationship exists between histamine and $PGF_{2\alpha}$. As $PGF_{2\alpha}$ rises above 60 pg/mL, an increase in histamine occurs in both the ACS and SCAD groups. A significant inverse relationship exists between ascorbate and histamine in the ACS versus C groups ($P < 0.01$) and the SCAD versus C groups ($P < 0.01$).
Conclusion: Histamine and isoprostane levels increase in SCAD and ACS patients. Mast cell activation and lipid oxidation generated during atherosclerosis manifest this inflammatory response. Accelerated isoprostane formation and depleted ascorbate paired with histaminemia is active in CAD and predispose patients to acute coronary syndrome. Blood histamine alone may be a better risk factor for coronary events, and a better prognostic indicator than CRP even when combined with lipid indexes.

Keywords: histaminemia - coronary artery disease syndrome - risk factors - oxidative stress - biochemical markers of inflammation - atherosclerosis

Introduction

Experimental studies by Clemetson indicated recently that low plasma ascorbic acid level and elevated blood histamine levels are involved in the development of atherosclerosis and coronary artery disease [1]. Low plasma ascorbate levels have been shown to be invariably associated with elevated blood histamine levels in guinea pigs [2] and humans [3]. Bacterial toxins and other forms of

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physical or chemical stresses putagens are also associated with elevated whole blood histamine levels, but may also be pertinent to developing vascular disease. In aortas of scurvyatic guinea pigs, diastasis or separation of endothelial cells from one another was observed by electron microscopic studies. [4]. Majno and Palade observed similar widening of intercellular junctions as the result of histamine infusion [5]. Histamine and neutral proteases are embedded in the proteoglycan matrix of secretory cells and mast cells. Histamine, a soluble component of intracellular granules, is easily released from these mast cells which promote progression of atherosclerosis from the early inflammation stage to the advanced lesion.

Histaminemia is characteristic of oxidative stress and may play a role in the formation of atherosclerosis. The role of the unstable atherosclerotic plaque in acute coronary syndrome is now well known. Plaque fissure, ulceration, and/or rupture have been shown to initiate the clinical state of acute coronary syndrome. Elevated histamine levels promotes progression of the atherosclerotic plaque and possibly accelerate its rupture, whereas, low ascorbate levels in animals appear to facilitate hemorrhage into the plaque [5].

The hemorrhage of clinical scurvy appears due to perivascular collagen instability superimposed on endothelial damage induced by histaminemia, while coagulation studies remain normal [6]. Vitamin C deficiency causes hemorrhage rather than thrombosis, however thrombosis is the normal mechanism for the arrest of hemorrhage. Intimal hemorrhages have been observed beneath thrombi in 52 out of 58 recently thrombosed coronary arteries, with hemosiderin deposits in the deep layers of the coronary arteries [7].

Clinical scurvy is quite rare in the Western World; but recent studies have shown that ascorbate-dependent histaminemia is relatively common [3, 8]. Furthermore, a recent eight-year study of 8,700 female nurses in Boston, MA has shown that the risk of coronary heart disease is significantly higher in women with low vitamin C intake [9]. There is a known inverse relationship between lipid oxidation and ascorbic acid; however, the relationship of blood histamine and lipid oxidation has not been adequately studied.

Oxidative stress which has emerged as an important co-factor in the development of endothelial dysfunction and atherogenesis, occurs in the presence of an excessive free radical production or low anti-oxidant level. In a study of atherosclerosis in chronic renal failure, vitamin E deficiency was strongly associated with an increased carotid intima media plaques area and these plaques were associated with log "oxidized LDL" in a multivariate logistic regression model [10]. Thus, a correlation exists not only between vitamin C, but also vitamin E levels and oxidized LDL; this may indicate that progressive atherosclerosis can be caused by a synergism of different mechanisms [11].

The goals of this study are to investigate the relationship between ascorbic acid and histaminemia in coronary heart disease, and to relate these to a marker of "oxidative" stress such as isoprostane and common lipid markers. We also wanted to examine our hypothesis that histaminemia may be a better marker of "active ulcerated plaque" than other markers of inflammation such as high sensitivity C reactive protein.

Methods

Study groups

Three groups of patients were investigated (n=50 in each group). *Group I* included patients with stable coronary heart disease (SCHD) as determined by clinical history, ECG, and coronary angiography, stress echocardiography, or nuclear stress testing. *Group II* included patients with an acute coronary syndrome (ACS) as determined by typical accelerating symptoms, acute electrocardiographic changes and serum enzymes troponin I, CK-MB, and/or myoglobin. *Group III* included age and sex matched healthy subjects. Patients in Group I and Group III were placed in the General Clinical Research Center (GCRC) overnight to ensure a state of minimal mental and physical stress, given a balanced dinner at 6:00 p.m., followed by a full night sleep and rest. It is well known that blood histamine levels vary greatly at time of stress. Blood was drawn at 8:00 a.m., after 14 h fasting, to prevent ingestion of vitamin C or E rich foods or supplements. In patients with acute coronary syndrome (Group II) the blood specimens were obtained fasting within the first 24 h of their acute event, after

initial medical stabilization in the CCU. Informed consent approved by the Institutional Review Board (IRB) was obtained from each subject. The GCRC research fellow (SSH) recorded a detailed clinical history and activity inventory. Data points included sex, age, inventory of dietary intake of vitamins and minerals, and smoking habits, history of exercise, illness, work activities, sleep activity, pain, recent inoculation, coryza, work and home stress level and other observations, such as anger, fear, agitation or nervousness. Particular emphasis was placed on existence of complicating factors such as uncontrolled diabetes mellitus, recent onset atrial fibrillation, recent upper respiratory infection, recent inoculation, sleep deprivation, recent accident or surgery.

Biochemical investigations

The biochemical measurements were performed blinded by laboratory personnel unaware of the clinical data. Heparinized whole blood sample (0.5 ml) was incubated with 9.5 ml of hypotonic medium (pH 5.0) for 30 min. to release the histamine. Released histamine in the supernatant is subsequently determined by specific whole blood competitive immunoassay, the histamine ELISA kit (IBL, Hamburg) using plasma-standards and control plasma. 50 ul sample was first acylated and then followed using the basic principle of the competitive ELISA. Histamine levels were quantitated exactly as per manufacturers' recommendation by reading the optical density at 450 nm within 60 minutes after stopping the reaction. The concentrations of the standards are plotted against their corresponding optical density on a semilogarithmic graph, and concentration of the samples were read directly from this standard curve.

For estimation of ascorbic acid, blood samples were centrifuged at room temperature at 1,500 X g for 5 minutes and plasma was transferred into two volumes of 20 g metaphosphoric acid (MPA)/L. For estimation of total vitamin C, dithiothreitol (DTT) was added to plasma at a concentration of 18 g/L before the addition of MPA. MPA extract was heated for 2 h at 45°C before HPLC analysis to convert all the dehydroascorbic acid to ascorbic acid. HPLC analysis was by Dhariwal method [12] and compared with the fluorimetric technique of Vuilleumier [13] which was used in most cases. (Correlation coefficient between the two techniques for 100 samples was r = 0.949). Recovery of total vitamin C was 101%. For lipid peroxidation *in vivo* we used the F_2-isoprostane iso $PGF_{2\alpha}$. Iso $PGF_{2\alpha}$ was first extracted from plasma by a one-step solid-phase procedure with a Waters Oasis cartridge containing a copolymer sorbent as described by Zhao [14]. The isoprostanes obtained by this purification procedure were analyzed by gas-chromatography-negative ion chemical ionization-mass spectroscopy. The results for recovery and precision performance were compared with the method of Nourooz-Zadeh [15] with a recovery of 88% vs. 70% and intra-assay cv of 5-5.5%. IL-6 (kit from Amersham), vitamin E, cholesterol, triglycerides and HDL were determined by automated methods in the routine hospital laboratory. Lipoprotein electrophoresis was used for LDL, VLDL and Lp (α) cholesterol determinations (Helena Laboratories), High sensitivity CRP (hsCRP) was performed by latex enhanced immunonephelometry (Dade-Behring) and diabetes control status was assessed by hemoglobin Ale with a variant II chromatographic system (Bio-Rad). Intra-assay coefficients of variation were < 5% for all other routine analytes.

Statistics

Values are expressed as mean + SD or median and interquartile ranges. Statistical differences were evaluated with the Wilcoxon test for comparison between 2 groups, the Kruskal-Wallis and Friedman test for > 2 groups or the % χ test. Correlation between variables were examined by Spearman rank correlation coefficients. Stepwise multivariate analysis was performed with histamine as the dependent variable. Statistical analysis was carried out with SPSS 9.0 software. Probability values < 0.05 were considered statistically significant.

Results

Clinical characteristics

Groups I, II and III did not differ in age, sex, or smoking habits, but other recognized risk factors and medication had a differential distribution (Table 1). Hypertension and adult onset diabetes mellitus was more common in Group I (SCHD) and Group II (ACS) patients than in controls. Cigarette smoking, however, was seen in about 1/3 of patients

Table 1. Clinical characteristics of study groups

	CONTROLS	SCHD	ACS
	n = 50	n = 50	n = 50
Age	62 ±12	61 ± 14	64 ± 12
Male sex %	54	55	58
Post-menopausal % of women	90	89	92
Body mass index (kg/m2)	25 ± 3	29 ± 6	28 ± 6
Hypertension %	0	30	45
Diabetes mellitus %	0	16	19
Smoking %	32	28	30
Vitamin C intake % (% taking at least 120 mg/day)	44	48	40
Physical activity, MET h/wk (metabolic units × duration h/w)	~ 25	~ 28	~ 20
Alcohol intake (g/week)	72	60	78
Coffee intake (g/day)	645 ± 34	520 ± 27	620 ± 38

Table 2. Pro-inflammatory markers, vitamin C and E levels in patients with SCHD or ACS vs. normal controls

	HISTAMINE	IL-6	HSCRP	VITAMIN C	VITAMIN E
	ng/ml	pg/ml	mg/L	mg/dL	mg/dL
C	38.8 ± 8.7	2.50 ± 1.2	0.92 ± 0.95	1.56 ± 0.29	1.30 ± 0.39
SCAD	73.6 ± 11.1a	3.74 ± 1.5b	1.44 ± 1.10	1.13 ± 0.20b	1.12 ± 0.34
ACS	84.5 ± 10.9a	3.61 ± 1.4b	3.09 ± 1.8b	0.80 ± 0.16a	0.65 ± 0.40b

Values are mean ± SEM:
a) $p < 0.01$
b) $p < 0.05$

in each group. Vitamin C, Vitamin E, and coffee intake were equally prevalent in each group, although coffee intake tended to be lower in the SCHD group. Physical work activity was equal in all groups. Total cholesterol, HDL and LDL cholesterol was similar in all groups. However, Lp (α) cholesterol tended to be higher in Group I and II versus control.

Medication usage differed as expected. β-blocker use was highest in SCHD (40%), intermediate in ACS (20%), and not used in controls. Calcium antagonists use was highest in SCHD (40%), intermediate but low in ACS (15%) versus none in controls. Similarly ACE inhibitor use was highest in SCHD (35%), intermediate in ACS (22%), and not in control.

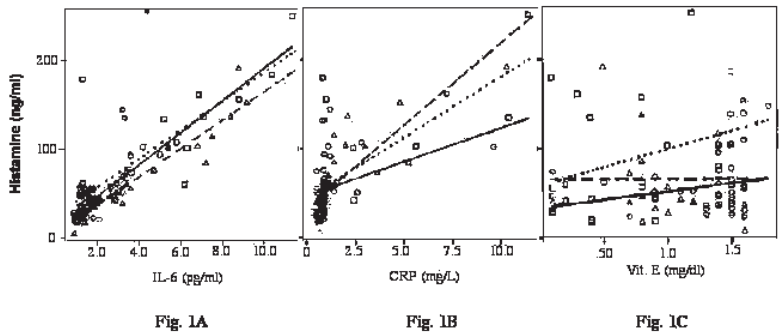

Fig. 1 Linear Regression:
a) IL-6 is independently related to histamine;
b) hsCRP is independently related to histamine;
c) Vitamin E is not independently related to histamine

Aspirin found its use in 90% of SCHD, 30% of ACS, 10% of controls. HMG CoA reductase inhibitor use was low in all groups but highest in SCHD (28%) and low in ACS and controls.

Biochemical investigations

ACS patients (Group II) had more than twice the blood concentration level of histamine and hsCRP than controls (Table 2). Also, SCHD patients had a significant increase in histamine and IL-6, although the increase in hsCRP was not significant. Both vitamin C and vitamin E were significantly lower in ACS patients (Group II) when compared with controls; SCHD (Group I) patients had a slight but significant decrease in vitamin C but not in vitamin E (Table 2).

Relation between histamine and inflammatory indexes or vitamin E or C

IL-6 and hsCRP were independently related to histamine (Fig. 1a & b) with a similar correlation with IL-6 for controls ($r^2 = 0.63$), SCHD ($r^2 = 0.89$), ACS ($r^2 = 0.67$), more defined but poorer correlation with hsCRP (controls, $r^2 = 0.11$; SCHD, $r^2 = 0.69$; ACS, $r^2 = 0.55$). No correlation was found between histamine and vitamin E ($r^2 = 0$ for SCHD; $r^2 = 0.1$ for ACS or 0.04 for controls) (Fig. Ic). Serum ascorbic acid concentration showed a negative correlation with blood histamine (Fig. 2). After adjustment for cigarette smoking and sleep stress a relative risk for vitamin C deficiency of 0.9 mg/dL (95% CI, 0.74 to 1.62) was calculated among controls with a blood histamine > 60 mg/ml (median value) and the equation for correlation (histamine = $59.8 – 84.9x + 58.9x^2 – 13.7x^3$) showed a r^2 of 0.92.

Serum vitamin C levels in the SCHD group with histamine concentrations > 60 mg/ml were comparable to those in the ACS group with a very rapid increase in histamine for every 1 mg/dL of vitamin C decrease. The equation values: $268.4 – 5200x + 349.7x^2 + 76.3x^3$ = histamine has an excellent r^2 of 0.97. This relationship remains true for patients irrespective of amounts of vitamin C intake.

Lipids and isoprostanes

Serum levels of LDL cholesterol were higher in less than 50% of patients in SCHD or ACS groups (Table 3). Two patients in each group had increases in Lp (α) without any other lipid abnormality. Homocysteine was only slightly increased in 6-9% of the patients with both SCHD and ACS. Decreased HDL was mostly associated with increases in LDL or Lp (α). Only 1 patient in the SCHD group, and 2 in the ACS group had low HDL without any other lipid abnormalities.

$8isoPGF_{2a}$ is an isoprostane which proved to be a reliable index of free radical oxidant stress. 72% of patients from the SCHD group and 82% of patients from the ACS group had high $8isoPGF_{2\alpha}$ (Table 3). $PGF_{2\alpha}$ were extremely high in the ACS group (400 pg/mL).

Table 3. Number of Patients with Hyperlipidemia vs. Increased 8-isoPGF$_{2\alpha}$

	LDL	HDL	Lp (a)	Homoycystine	8-isoPGF$_{2\alpha}$
SCAD	22	16	12	3	36
ACS	24	22	10	4	41

Hyperlipidemia: Patients with LDL cholesterol > 135 mg/dL, HDL < 40 mg/dL, Lp (α) >10 mg/dL, Homocysteine > 15 mg/dL and 8-isoPGF$_{2\alpha}$ > 20 pg/mL

Correlations with 8-isoPGF$_{2\alpha}$,

Fig. 3 shows a relative the good correlation between vitamin E and isoPGF$_{2\alpha}$, (r^2 = 0.88-0.95), although no significant differences are seen between different groups. Fig. 4 illustrates the negative correlation of isoPGF$_{2\alpha}$ with vitamin C with the same correlation for SCHD, ACS and control groups to vitamin C < 1.0 mg/dL, and controls with a lower increase in 8isoPGF$_{2\alpha}$ than SCHD patients. The lower the vitamin C, the higher values for iso PGF$_{2\alpha}$ were demonstrated in ACS group. The three curves could be fitted with equations with excellent correlation coefficients (r^2 = 0.995-0.997) (Fig. 4). The positive correlation between histamines and iso PGF$_{2a}$ (Fig. 5) shows the same correlation coefficient (r^2 = 0.92-0.96) with different increases in PGF$_{2\alpha}$ above a histamine of 60 mg/ml, for patients in groups 1 and 2 versus controls.

Discussion

This study suggests that histamine may be associated with active coronary artery disease. Table 2 convincingly shows higher levels of histamine in SCHD patients when compared with matched for age, sex and other confounding factors (smoking, lipids, physical activity) controls. Even statistically significant higher levels of histamine are found in ACS patients. So, histamine is related not only to the presence but also the extent of atherosclerosis, and with cardiac events during follow-up. The positive good correlation between hsCRP and histamine shows also that histamine is a marker of subclinical inflammation. This is enhanced by demonstration of higher circulating levels of pro-inflammatory atherogenic cytokine, IL-6 in CAD stable or acute patients.

Cytokine-activated monocyte/macrophages produce increased amounts of IL-6, which may enter the systemic circulation and lead to increased production of CRP by hepatocytes [16] and both increase even further the histamine. The biphasic inverse correlation between histamine and vitamin C was first found in normal controls by Clemetson [1, 3]. The important finding of this study was that this biphasic negative relation holds for SCHD or ACS patients. Until a blood histamine of 60 mg/ml is achieved, the relation with vitamin C is the same for all three groups; above this level ACS and SCHD patients show a higher increase in histamine for each mg/dL of vitamin C. This relation is sufficient (r^2 = 0.97) to be able to predict the vitamin C concentration from the values of histamine. This is important due to the fact that analysis of vitamin C in plasma is cumbersome, and not very precise by any other biochemical assay except HPLC.

Detailed information of vitamin supplement intake from all subjects showed that even with high dietary intake of vitamin C, once histamine increased more than 60 mg/ml, the plasma vitamin C decreased below 0.9 mg/dL which is the value from which we can consider definite vitamin C deficiency. Ascorbate is considered to be the most effective antioxidant in human plasma [17], and vitamin C inhibits the oxidation of low density lipoprotein in vitro [17–19]. Ascorbic acid is the first antioxidant to be used up during lipid peroxidation in plasma, and detectable lipid peroxidation starts only after all ascorbate has been completely used up; Frei [20] and others [17] have even suggested that only ascorbate can prevent the initiation of lipid peroxidation. Ascorbate is an important physiological antioxidant that helps to regenerate reduced antioxidative tocopherol from the toco-

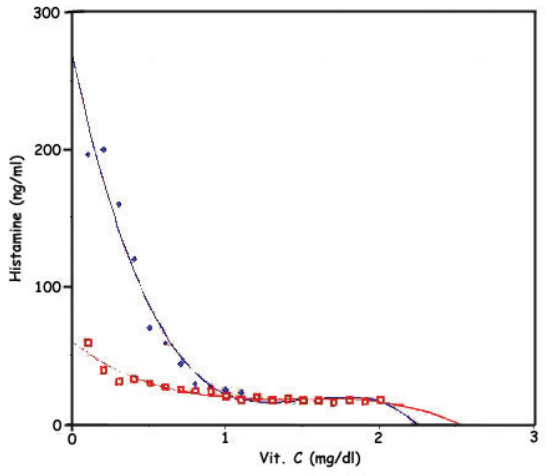

Fig. 2 Serum Vitamin C shows a negative correlation with histamine

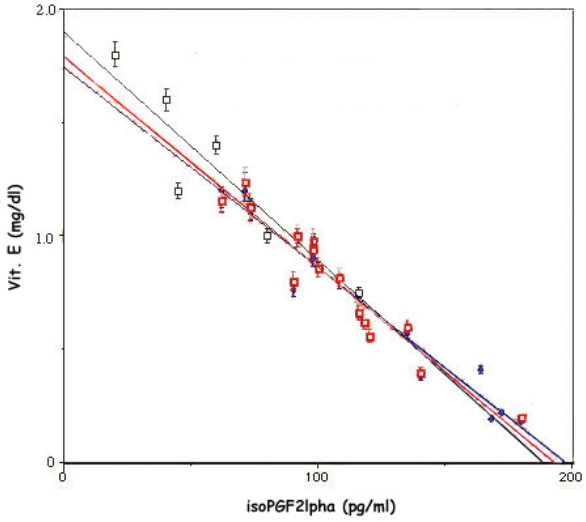

Fig. 3 Vitamin E shows good correlation with isoPGF$_{2\alpha}$

pheroxyl radical [20]. Although it is not lipid soluble, ascorbate could theoretically also inhibit lipid peroxidation through this mechanism. Retsky and Frei have suggested that vitamin C spares, rather than regenerates a tocopherol and other endogenous antioxidants when low density lipoprotein is exposed to copper ions and that ascorbic acid can terminate lipid peroxidation, thereby protecting partially oxidized low density lipoprotein against further oxidative modification. Our results on vitamin E and the same correlation with histamine for controls as well as for SCHD or ACS confirms these hypotheses.

Riemersma *et al.* [21] observed an association between low plasma concentrations of vitamin C and an increased risk of angina in a population based case-control study. The unadjusted risk was 2.4 in the lowest fifth of plasma vitamin C concentration (< 13.1 μmol/l). This odds ratio was weakened to 1.6 and was not significant when smoking and other coronary risk factors were adjusted for.

In our study, the adjusted (for cigarette smoking and sleep stress) odds ratio was 2.2. Confounding is a major problem in all studies between nutrient intake and the risk of disease. We tried to assess differences between subjects with low and high plasma vitamin C concentrations in every respect that was relevant to coronary risk, however, some unmeasured factors may have confounded the relation or the statistical control for confounding may not be good enough due to inprecision in measurement. But beyond any statistical doubt high intakes of vitamin C, will not reduce the risk of ACS. Our findings suggest, instead, that if: **1)** a minimal necessary requirement of vitamin C is not met, the risk of a cardiac event is increased, and **2)** histamine may measure better the effect of inflammation on antioxidant levels than plasma vitamin C.

We observed the in vitro stability of vitamin C is low in serum from ACS patients with high histamine, suggesting a higher degree of oxidative stress associated with inflammation. Considerable evidence has implicated reactive free radicals, especially those derived from oxygen, in the pathophysiology of a wide spectrum of disorders including atherosclerosis, ischemia-reperfusion injury, inflammatory diseases, and aging. Peroxidation of lipids is a well-recognized sequela of oxidant injury but, despite the variety of methods devised to detect this phenomenon, reliable assessment of lipid peroxidation *in vivo* were problematic. In fact, this has been a major impediment to investigations in this field [22]. Previous evidence for enhanced lipid atherosclerosis was largely indirect and based on crude measurements of lipid oxidation products in plasma [23, 24] or the susceptibility of the patient's LDL to oxidation *in vitro* [25, 26]. Recently, reports of the discovery of a series of prostaglandin (PG)

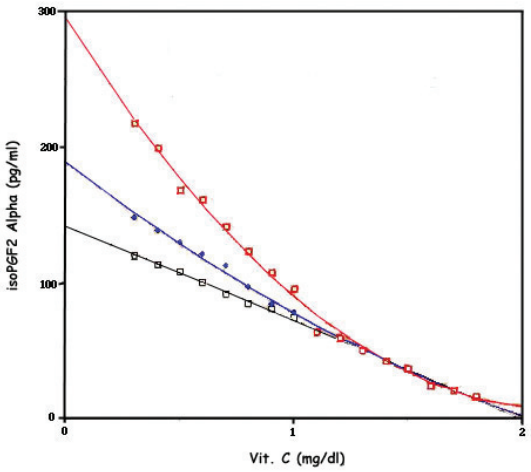

Fig. 4 Vitamin C shows a negative correlation with isoPGF$_{2\alpha}$

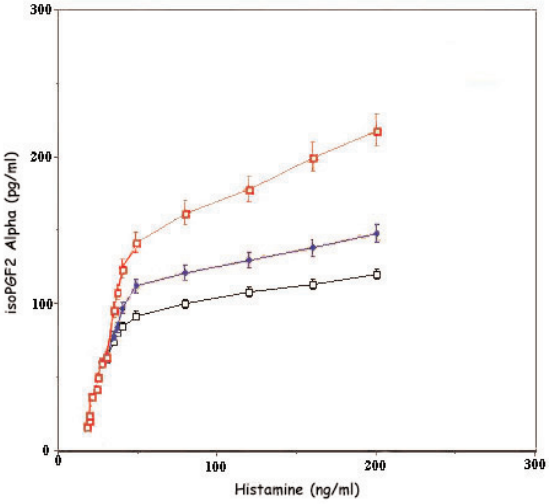

Fig. 5 isoPGF$_{2\alpha}$ shows good correlation with histamine.

■ Controls; ♦ SCAD ; ■ ACS

F_2-like compounds in humans that are produced *in vivo* by free radical catalyzed peroxidation of arachidonyl-containing lipids began to appear [27]. Formation of these compounds is independent of the catalytic activity of the cyclooxygenase enzyme. In normal human plasma and urine levels of unmetabolized F_2-isoprostanes exceed those of unmetabolized cyclooxygenase-derived prostaglandins by approximately an order of magnitude. In addition, plasma F_2-isoprostane levels have been found to increase up to 500-fold in two animal models of free radical injury and lipid peroxidation, administration of diquat to selenium-deficient rats and $CC1_4$ to normal rats. These results suggest that quantification of endogenous production of F_2-isoprostanes may provide a valuable tool to assess oxidant stress *in vivo*. Our study used the plasma F_2-isoprostane 8-iso PGF$_{2\alpha}$ as a marker of in vivo lipid peroxidation.

We found that the formation of 8-iso PGF$_{2\alpha}$ was abnormally elevated in the vast majority of both SCHD and ACS patients, carefully characterized for other variables potentially influencing 8-iso PGF$_{2\alpha}$. Thus, we excluded the contribution of advanced age by adequate age-matching of individual patients and control subjects. As shown in Table 3, less than 50% of patients had high cholesterol, LDL, more patients had Lp (α) increase. The Lp (α) correlated well with decreased HDL in few patients. But more than 70% patients in the SCHD group and 80% of patients in the ACS group had high to extremely high PGF$_{2\alpha}$ We also found a high significant correlation between 8-isoPGF$_{2\alpha}$ and histamine or vitamin C (Fig. 4 and 5). And we could stratify patients in groups based on high PGF$_{2\alpha}$ above 80 ng/ml. On the basis of these findings, it is tempting to speculate that changes in the rate of arachidonate peroxidation to form biologically active iso-eicosanoids, such as 8-iso PGF$_{2\alpha}$ may represent an important biochemical link between altered or increased histamine, oxidant stress, platelet activation, peroxidation burden, immune response to stress, proinflammatory atherogenic cytokines, and endogenous antioxidants, in both SCHD or ACS patients. The important role histamine plays in initiating thrombosis and vasoconstriction has been studied in animal models [5]. Factors associated with coronary artery disease and acute events are probably not exclusively either thrombogenic or atherogenic; there is evidence to support both effects for histamine.

In conclusion, we found evidence that high total blood histamine is associated with confirmed CAD and subsequent acute cardiac events and may prove

to be a single excellent marker for atherosclerosis and coronary events. Blood histamine testing may increase the yield of programs designed to detect high-risk patients for subsequent coronary occlusion, in the setting of global risk-assessment programs designed to better target intervention efforts.

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