

Mast cell tryptase: a new biomarker in patients with stable coronary artery disease

Efthymios N. Deliargyris^{a,b}, Bharathi Upadhy^a, David C. Sane^{a,*}, Gregory J. Dehmer^c, Joseph Pye^d, Sidney C. Smith Jr.^d, William S. Boucher^e, Theoharis C. Theoharides^{e,f}

^a Cardiology Section, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1045, USA

^b Athens Medical Center, Athens, Greece

^c Scott & White Clinic, Texas A&M School of Medicine, Temple, TX, USA

^d University of North Carolina School of Medicine, Chapel Hill, NC, USA

^e Department of Pharmacology and Experimental Therapeutics, Boston, MA, USA

^f Internal Medicine, Tufts University School of Medicine, Boston, MA, USA

Received 29 January 2004; received in revised form 29 July 2004; accepted 14 September 2004

Available online 8 December 2004

Abstract

Mast cells may participate actively in the inflammatory process of atherosclerotic plaques by releasing proteolytic enzymes and various other pro-inflammatory substances. We hypothesized that increased levels of mast cell tryptase, could be an important biomarker in patients with stable coronary artery disease (CAD). We measured tryptase in 102 patients without acute coronary syndromes undergoing cardiac catheterization. Patients with significant CAD [$\geq 50\%$ stenosis in ≥ 1 artery ($n = 66$)] had significantly higher serum tryptase than patients with normal angiography ($n = 13$) or non-significant CAD [$< 50\%$ stenosis ($n = 23$)]. The median, 25th and 75th percentiles for tryptase in these two groups were 8.38 (6.4 and 10.7) $\mu\text{g/L}$ versus 6.78 (5.61 and 9.72) $\mu\text{g/L}$, $p = 0.014$. Patients in the highest quartile of tryptase levels had a 4.3-fold risk for CAD [Odds ratio (OR): 4.3; 95% confidence interval (CI): 1.08–17.19; $p = 0.04$]. In a multivariate regression analysis, tryptase remained an independent predictor for CAD along with age (OR: 1.178; 95% CI: 1.021–1.359, $p = 0.025$). High circulating tryptase levels may be a result of chronic low-grade inflammatory activity present in atherosclerotic plaques. Tryptase measurements may emerge as a novel way of identifying asymptomatic patients with CAD, and represent a new biomarker of therapeutic efficacy in patients with CAD. © 2004 Elsevier Ireland Ltd. All rights reserved.

Keywords: Mast cells; Tryptase; Coronary artery disease

1. Introduction

Atherosclerosis is a complex disease characterized by smooth muscle proliferation and migration, cholesterol deposition, infiltration of mononuclear cells and increased extra cellular matrix production [1,2]. Mast cells (MC) have been consistently demonstrated as part of the inflammatory response within atherosclerotic plaques [3]. Their density is markedly higher in the shoulder region of atherosclerotic plaques, particularly in coronary atheroma [4–6]. Matrix

degradation within the atherosclerotic plaque is responsible for weakening and ruptures of plaque [7,8]. Tryptase and chymase are two proteolytic enzymes, released by MC that directly degrade certain components of the peri-cellular matrix. For example, fibronectin is proteolyzed by tryptase in a dose-dependent manner [9]. These enzymes can also activate matrix metalloproteinases (MMPs), which further degrade the extracellular matrix [10,11]. Tryptase activates pro-MMP-3 that in turn can activate MMP-1 (pro-collagenase); the activation of MMP-1 would require very little tryptase because of this cascade effect [12,13]. Acting via this mechanism, MC and its mediators may play an important role in unstable plaque rupture in acute coronary syndromes [4,14].

* Corresponding author. Tel.: +1 336 716 7533; fax: +1 336 716 9188.
E-mail address: dsane@wfubmc.edu (D.C. Sane).

In addition to a role in plaque rupture, it is conceivable that chronic mast cell activation within atherosclerotic plaques would lead to elevated serum tryptase levels. We measured tryptase levels in patients without acute coronary syndromes undergoing catheterization and compared levels in patients with and without obstructive CAD.

2. Methods

2.1. Study population and study groups

Serum tryptase was measured in 102 patients undergoing cardiac catheterization with left ventriculography and coronary angiography for a variety of clinical indications including chest pain evaluation ($n = 48$), positive non-invasive testing (38) and initial evaluation of heart failure ($n = 16$). There were no specific inclusion criteria other than a willingness to provide informed consent. Exclusions included a history of cardiac transplantation, ongoing treatment with corticosteroids and recent (<3 months) acute coronary syndrome (ACS) (unstable angina, non-Q-wave and Q-wave myocardial infarctions). The Institutional Review Board of the medical school approved the study protocol. The study population was divided into two groups: patients with significant CAD [$\geq 50\%$ stenosis in ≥ 1 artery ($n = 66$)] and patients with normal angiography ($n = 13$) or non-significant CAD [$< 50\%$ stenosis ($n = 23$)].

2.2. Cardiac catheterization

Coronary arteriography was performed in multiple right and left anterior oblique projections with cranial and caudal angulations for visualization of all segments of the coronary arteries. Stenosis severity was determined by the consensus reading of two experienced angiographers, who were blinded to risk factors. Biplane left ventriculography was performed in a 50° left anterior oblique and 30° right anterior oblique projection. Left ventricular volumes and ejection fractions (LVEF) were calculated by the biplane area-length method with the Wynne regression formula.

2.3. Definitions

Significant CAD: At least one stenosis $\geq 50\%$ diameter narrowing in at least one of the three major epicardial coronary arteries.

Non-significant CAD: The presence of any luminal irregularity ($< 50\%$) by angiography. Clinical congestive heart failure (CHF) was assigned to patient with \geq NYHA class-II symptoms. Peripheral arterial disease (PAD) included stroke documented by computed tomography or magnetic resonance imaging or persistent focal neurologic deficit, carotid endarterectomy, peripheral vascular surgery or positive noninvasive lower arterial studies. Diabetes, hypertension and hyperlipidemia were defined by a previous medical

diagnosis, or the use of medications or fasting blood sugar > 126 mg/dl, or admission blood pressure $> 140/90$ mmHg or fasting total cholesterol > 200 mg/dl. All patients had a complete blood count measured as part of routine pre-procedural protocol. The pre-procedural white blood cell (WBC) count obtained the closest to the angiography was used in patients with more than one value on record.

2.4. Sampling and tryptase measurement

Samples were obtained from the femoral artery after sheath insertion before heparin or contrast administration. Samples were allowed to clot and then centrifuged at room temperature for 5 min. The serum was removed and frozen at -80°C for analysis at a later date. Tryptase measurements were performed by radioimmunoassay (Uni Cap[®], Pharmacia, Kalamazoo, MI) and values are expressed in $\mu\text{g/L}$.

2.5. Statistical analysis

Continuous variables are expressed as mean \pm standard deviation and comparisons are made with unpaired *t*-test for normally distributed data. Because of extremely elevated tryptase levels in certain patients, the data were not normally distributed. All tryptase values are, therefore, expressed as the medians with 25th and 75th percentiles and comparisons are made with Mann-Whitney rank sum test. Chi-square analysis or the Fisher exact test, where appropriate, was used for categorical parameters. Correlation of the association between tryptase and CAD was done with Spearman's rank correlation and expressed as the correlation coefficient and tested for significance using Spearman's test for correlation. We stratified tryptase into quartiles (Q_1 – Q_4) and compared base line characteristics among these. We calculated the Cox proportional Odds ratio (OR) and 95% confidence interval (CI) for the upper three quartiles of tryptase as compared to the lowest quartile for CAD. Multivariate logistic regression analysis was used to determine independent variables for the presence of CAD {tryptase, age (expressed as categorical variable), male gender, hypertension, hyperlipidemia, diabetes, PAD, smoking, body mass index (BMI) and pre-procedural WBC count}. Forward, backward, and stepwise techniques were used for the multivariate regression model. The Kruskal-Wallis test was used to determine the association between the five groups in age and tryptase. A *p*-value < 0.05 was considered as significant. Statistical analysis was done with SIGMASTAT version 2 statistical software (SPSS Inc., Chicago, IL).

3. Results

3.1. Patient characteristics

The mean age of the study population was 59.7 ± 12.2 years. There were 69 (67.6%) males. The tryptase ranged from 1.95 to 34.8; median and 25th, 75th percentiles were

Table 1
Base line characteristics and admission medications between two groups

	CAD (n = 66)	No CAD (n = 36)	p-value
Age (years)	62.9 ± 11.8	53.9 ± 10.7	<0.001
Male (%)	43 (65.2)	26 (72.2)	NS
Caucasian (%)	56 (84.9)	23 (63.9)	0.03
Diabetes (%)	23 (34.9)	7 (19.4)	0.16
Hypertension (%)	43 (65.2)	23 (63.9)	NS
Hyperlipidemia (%)	29 (43.9)	13 (36.1)	NS
Smoking (%)	34 (51.5)	21 (58.3)	NS
Prior congestive heart failure (%)	9 (13.6)	7 (19.4)	NS
Peripheral arterial disease (%)	9 (13.6)	1 (2.8)	0.160
Body mass index (%)	29.1 ± 6.2	30 ± 6.3	NS
Aspirin (%)	54 (81.8)	22 (61.1)	0.04
ACE-I (%)	26 (39.4)	11 (30.6)	NS
Statins (%)	11 (16.7)	9 (25)	NS
LVEF (%)	60.8 ± 18.6	58 ± 17.8	NS
LVEDV (mm ³)	84.1 ± 28.8	87.4 ± 31.9	NS
ESR (cm/h)	23.0 ± 16.9	21.3 ± 19.1	NS
Pre-procedural WBC count (× 10 ³ cells/mm ³)	8.19 ± 2.45	7.19 ± 2.18	0.044

CAD, coronary artery disease; NS, nothing significant; ACE-I, angiotensin converting enzyme; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; ESR, erythrocyte sedimentation rate; WBC, white blood cells.

7.93 (6.1–10.0) µg/L. Baseline characteristics between the two groups are shown in Table 1, demonstrating that patients with CAD were older ($p < 0.001$), predominantly Caucasian ($p = 0.03$), more often received aspirin ($p = 0.04$) and had higher pre-procedural WBC count ($p = 0.04$).

3.2. Tryptase and CAD

The median, 25th and 75th percentiles of tryptase levels were significantly higher in patients with CAD [8.38 (6.4–10.7) µg/L versus 6.78 (5.6–9.72) µg/L; $p = 0.014$, Fig. 1]. The Spearman's rank order correlation between tryptase (continuous variable) and CAD supported a significant positive association ($r_s = 0.27$, $p = 0.014$). We stratified the serum levels of tryptase into quartiles and examined the base line characteristics among these quartiles. Age, preva-

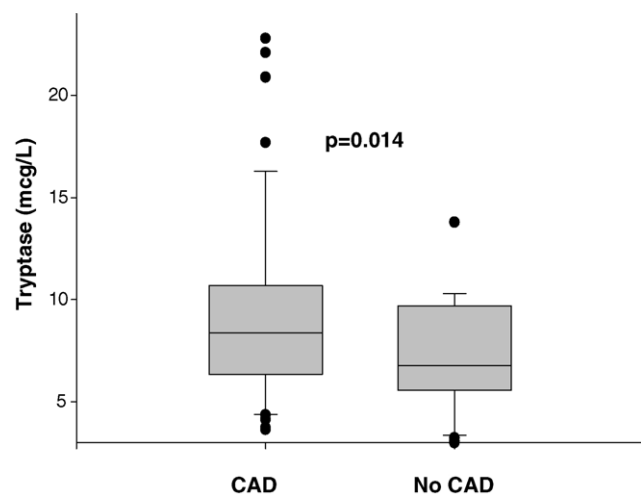


Fig. 1. A box plot showing the median, 25th and 75th percentiles for tryptase (mcg/L) of two groups based on CAD.

lence of CAD and ACE inhibitors use were significantly higher in Q₄ with respect to Q₁ ($p < 0.05$) (Table 2). The Q₄ was associated with a fourfold increased risk of CAD as compared to Q₁ [Q₂:Q₁, OR: 1.73, 95% CI: 0.36–8.21, $p = ns$; Q₃:Q₁, OR: 2.44, 95% CI: 0.61–9.8, $p = ns$; Q₄:Q₁, OR: 4.3, 95% CI: 1.08–17.19, $p = 0.04$]. Finally, in a multivariate regression analysis with stepwise, backward and forward logistic regression, tryptase and age (>65 years) were two independent variables that associated with CAD (Table 3).

3.3. Tryptase and PAD

Even though serum tryptase levels were positively correlated with CAD, PAD was not independently associated with an increase in serum tryptase levels [8.0 (6.12–10.05) µg/L versus 6.65 (6.1–9.2) µg/L; $p = 0.458$]. The Spearman's rank order correlation between tryptase and PAD did not support a positive association ($r_s = -0.08$, $p = 0.456$). There was no progressive increase in prevalence of PAD with quartiles of tryptase (Table 2).

3.4. Tryptase and age

Patients in the CAD group were significantly older than the no CAD group (62.9 ± 12 versus 53.9 ± 11 ; $p < 0.001$). (Table 1) A linear regression analysis showed a positive correlation between tryptase and age ($r^2 = 0.05$, $p = 0.019$). To further delineate the effects of age on tryptase we divided the age into five groups (<40 years, 40–49 years, 50–59 years, 60–69 years and >70 years). Comparison was made between these five groups for tryptase with the Kruskal–Wallis test, but there was no significant difference among these groups ($p = 0.28$; Fig. 2).

Table 2
Base-line characteristics for quartiles of tryptase

	Q ₁ (n = 26)	Q ₂ (n = 25)	Q ₃ (n = 27)	Q ₄ (n = 24)
Tryptase (µg/L)	5.05 (3.65–5.74)	6.9 (6.48–7.31)	9.1 (8.46–9.78)	11.35 (10.6–16.7)
Age (years)	54.7 ± 11.3	59.8 ± 12.8	60.5 ± 12.1*	61.1 ± 16.1*
Sex (%)	19 (76)	17 (68)	18 (66.7)	15 (60)
Race (%)	20 (80)	15 (60)	23 (85.1)	21 (84)
Coronary artery disease (%)	12 (48)	15 (60)	19 (70.4)	20 (80)*
Diabetes (%)	5 (20)	7 (28)	8 (29.6)	10 (40)
Hypertension (%)	14 (56)	19 (76)	15 (55.6)	18 (72)
Hyperlipidemia (%)	7 (28)	15 (60)*	11 (40.7)	9 (36)
Smoking (%)	13 (52)	17 (68)	12 (44.4)	13 (52)
Body mass index (%)	28.1 ± 7.1	29.8 ± 5.6	28.2 ± 3.7	31.6 ± 7.6
Peripheral arterial disease (%)	3 (11.5)	3 (12)	2 (7.4)	2 (8.3)
Congestive heart failure (%)	6 (23.1)	5 (20)	1 (3.7)	4 (16.7)
Statins (%)	2 (7.7)	7 (28)	8 (29.6)	3 (12.5)
Aspirin (%)	17 (65.4)	21 (84)	18 (66.7)	20 (83.3)
ACE inhibitors (%)	5 (19.2)	9 (36)	11 (40.7)	12 (50)*
WBC count (×10 ³ cells/mm ³)	7.8 ± 2.6	7.6 ± 2.4	7.6 ± 2.4	8.2 ± 2.2

Q₁–Q₄, quartiles 1–4; ACE, angiotensin converting enzyme; WBC, white blood cell.

* $p < 0.05$ (compared with Q₁).

3.5. Tryptase and WBC

The pre-procedural WBC counts were higher in patients with CAD, but there were no significant differences in WBC count among the quartiles of tryptase. (Tables 1 and 2) Linear regression between tryptase (dependent variable) and WBC count failed to show a positive correlation ($r^2 = 0.002$, $p = 0.6$). Based on the WBC levels, the patient population was divided into two groups (normal and increased WBC level). Again, there was no association between tryptase and WBC count [7.8 (6.0–10.0) µg/L versus 8.0 (6.3–10.3) µg/L; $p = 0.68$].

3.6. Tryptase and heart failure

Among the 16 patients who underwent catheterization for initial evaluation of heart failure, CAD turned out to be the etiology in nine (56%) patients. There was no difference in serum tryptase level between patients with

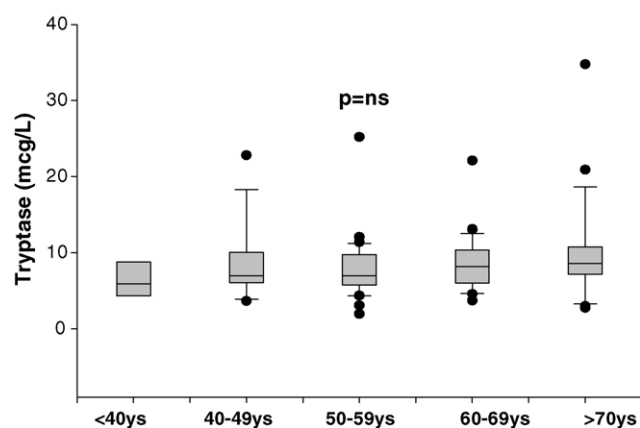


Fig. 2. A box plot showing the median, 25th and 75th percentiles for tryptase (mcg/L) of five different age groups.

Table 3
Multivariate logistic regression analysis for CAD

Independent variables	Odds ratio (95% CI)	<i>p</i> -value
Tryptase (µg/L)	1.178 (1.021–1.359)	0.025
Age (>65 years)	1.066 (1.017–1.116)	0.007
Male	0.981 (0.346–2.778)	0.970
Diabetes	2.255 (0.726–7.003)	0.160
Smoking	1.061 (0.398–2.830)	0.905
Hypertension	0.739 (0.258–2.118)	0.574
Hyperlipidemia	1.326 (0.488–3.602)	0.580
Congestive heart failure	0.537 (0.128–2.249)	0.395
Peripheral arterial disease	8.466 (0.643–111.483)	0.104
Body mass index	0.978 (0.901–1.062)	0.604

CAD, coronary artery disease; CI, confidence interval.

CHF and those without CHF [6.63 (3.90–10.05) µg/L versus 8.07 (6.2–10.0) µg/L; $p = 0.184$]. Linear regression between tryptase and LVEF (%) failed to show any correlation ($r^2 = 0.005$, coefficient = 0.629, $p = 0.531$). There was no progressive increase in prevalence of heart failure with quartiles of tryptase (Table 2).

Moreover, even after the exclusion of CHF patients from the entire study population, patients with CAD had a significantly higher serum level of tryptase [8.5 (6.78–10.55) µg/L versus 6.9 (5.72–9.71) µg/L, $p = 0.03$].

4. Discussion

Our findings of elevated circulating tryptase in patients with CAD without clinical instability compliment the wealth of reports evaluating the systemic profile of inflammatory mediators in the pathogenesis of atherosclerosis. Although elevated markers of activated mast cells have been previously described in patients with unstable coronary syndromes [4,14], elevated serum levels of tryptase have not previously been demonstrated in patients with stable coronary artery disease.

There are many reports of several elevated acute phase inflammatory mediators in unstable coronary syndromes [15–17]. Elevations of inflammatory markers including CRP, IL-6, serum amyloid protein A and fibrinogen have been reported in patients with angiographically proven stable CAD [18–20]. In contrast, only a few studies have measured elevated serum levels of tryptase with patients with ACS [21–23]. Filipiak et al. [21] observed elevated serum tryptase levels with ACS with ST-depression both in the acute phase and 3 months later, but Van Haelst et al. [23] showed no elevation of serum tryptase with ACS. In our study, we excluded all ACS (<3 months) patients and we clearly could not find a positive association between tryptase and CAD. Tryptase was significantly associated with CAD not only as a categorical but also as a continuous variable. In a multivariate regression model with CAD as a dependent variable, increased tryptase was an independent predictive marker for CAD. In addition, patients with the highest quartile levels of tryptase also tended to be older and used more ACE inhibitors.

Increased serum tryptase with increased age can be explained by the fact that the patients with CAD were significantly older since there was no significant difference in tryptase level among the different age groups. We could not find any association of serum tryptase with LVEF and pre-procedural WBC count.

4.1. Explanations for increase tryptase in stable CAD

Previous studies have reported a striking increase in the number of activated mast cells at the sites of thrombotic atheromatous erosion and the presence of mast cells at the site of erosion/rupture before ischemic episodes [6]. Mast cell density was found proportional to the severity of the atheroma. In addition to this, pathologic studies in cardiomyopathy have demonstrated higher MC numbers in failing hearts secondary to ischemic compared to a nonischemic etiologies and higher numbers in akinetic rather than non-akinetic segments [24,25]. When and how were these mast cells activated? There are number of potential mechanisms, including being IgE mediated or stimulated by other immunological activated cells, such as T lymphocytes or macrophages [26–28]. Actually, mast cells were found to be accompanied by T lymphocytes and macrophages, at the sites coronary artery erosion or rupture and the degree of degranulation was highest at those sites where the numbers of other inflammatory cells were highest [4]. Recently, it has been shown that stress can trigger mast cell activation [29]. Our finding of elevated tryptase levels could be due to ongoing mast cell activation, degranulation and possibly plaque inflammation. The higher systemic tryptase levels in these patients may be explained by the fact that MC has been consistently demonstrated within coronary plaques. However, it is possible that non-coronary plaques such as those in the peripheral arteries or aorta account for a preponderance of the circulating tryptase levels. This is an attractive possibility since the plaque volume in these vessels can be significantly

greater than the coronary, and they also contain mast cells and tryptase [5]. Thus, it is possible that the correlation between CAD and tryptase levels represents an underlying association between CAD and PAD, with the latter being the predominant source of tryptase.

4.2. Implications

Acute phase inflammatory markers, such as IL-6 and C-reactive protein are elevated with unstable coronary syndromes and appear to have prognostic significance [15–17]. However such markers are not helpful as screening tools for the presence of coronary artery disease or for the monitoring therapies targeting reduction of local inflammation and plaque stabilization. Tryptase measurements may have important clinical value as a screening test for CAD in healthy individuals, and as a means of monitoring the efficacy of therapies targeting plaque stabilization in patients with known CAD.

4.3. Limitations

Several limitations of our data merit consideration. The relatively small number of our patients included in this study is an important limitation. This small number limits our ability to examine tryptase levels in important sub-groups such as those with PAD. Although, we excluded all ACS patients, we do not have data on what percentage of these patients had distant ACS. Almost all our patients are Caucasian, limiting extrapolation of the data to other ethnic groups. We have measured tryptase only once and we have no evidence for alterations during the course of follow-up with progression of disease. Unfortunately, the current study design did not include multi-site sampling. We have not correlated the serum levels of tryptase with extent of disease. We did not measure the two forms of tryptase separately; hence we have not studied the influence of factors that determine the mast cell degranulation. It would have been very helpful to measure CRP and other inflammatory markers in these patients along with tryptase to compare the possible utility of those markers in monitoring therapies targeting for reduction of local inflammation and plaque stabilization.

We conclude that tryptase may serve as a novel inflammatory marker in patients with stable CAD. Based on our observations, we speculate that high circulating tryptase levels are probably the result of the chronic low-grade inflammatory activity present in atherosclerotic plaques. Tryptase measurements may emerge as a novel way of identifying asymptomatic patients with CAD, and as a measure of therapeutic efficacy in patients with CAD.

References

- [1] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801–9.

- [2] Ross R. Atherosclerosis: a defense mechanism gone awry. *Am J Pathol* 1993;143:987–1002.
- [3] Kelley JL, Chi DS, Abou-Auda W, Smith JK, Krishnaswamy G. The molecular role of mast cells in atherosclerotic cardiovascular disease. *Mol Med Today* 2000;6:304–8.
- [4] Kovanen PT, Kaartinen M, Paavonen T. Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation* 1995;92:1084–8.
- [5] Kaartinen M, Penttilä A, Kovanen PT. Mast cells of two types differing in neutral protease composition in the human aortic intima: demonstration of tryptase- and tryptase/chymase-containing mast cells in normal intimas, fatty streaks, and the shoulder region of atheromas. *Arterioscler Thromb* 1994;14:966–72.
- [6] Kaartinen M, Penttilä A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. *Circulation* 1994;90:1669–78.
- [7] Richardson PD, Davies MJ, Born GVR. Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques. *Lancet* 1989;2:941–4.
- [8] Falk E. Why do plaques rupture? *Circulation* 1992;86(suppl III):III-30–42.
- [9] Lohi J, Harvima I, Keski-Oja J. Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin 1992;50:337–49.
- [10] Jeziorska M, McCollum C, Woolley DE. Mast cell distribution, activation, and phenotype in atherosclerotic lesions of human carotid arteries. *J Pathol* 1997;182:115–22.
- [11] Kaartinen M, Penttilä A, Kovanen PT. Mast cells accompany microvessels in human coronary atheromas: implications for intimal neovascularization and hemorrhage. *Atherosclerosis* 1996;123:123–31.
- [12] Saarinen J, Kalkkinen N, Welgus HG, Kovanen PT. Activation of human interstitial procollagenase through direct cleavage of the Leu83-Thr84 bond by mast cell chymase. *J Biol Chem* 1994;269:18134–40.
- [13] Gruber BL, Marchese MJ, Suzuki K, et al. Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. *J Clin Invest* 1989;84:1657–62.
- [14] Kaartinen M, van der Wal AC, van der Loos CM, et al. Mast cell infiltration in acute coronary syndromes: implications for plaque rupture. *J Am Coll Cardiol* 1998;32:606–12.
- [15] Biasucci LM, Vitelli A, Liuzzo G, et al. Elevated levels of interleukin-6 in unstable angina. *Circulation* 1996;94:874–7.
- [16] Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in active coronary artery disease. *Am J Cardiol* 1990;65:168–72.
- [17] Mach F, Lovis C, Gaspoz JM, et al. C-reactive protein as a marker for acute coronary syndromes. *Eur Heart J* 1997;18:1897–902.
- [18] Hoffmeister A, Rothenbacher D, Bazner U, et al. Role of novel markers of inflammation in patients with stable coronary heart disease. *Am J Cardiol* 2001;87:262–6.
- [19] Rifai N, Joubran R, Yu H, Asmi M, Jouma M. Inflammatory markers in men with angiographically documented coronary heart disease. *Clin Chem* 1999;45:1967–73.
- [20] Johnson BD, Kip KE, Marroquin OC, et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation* 2004;109:726–32.
- [21] Filipiak KJ, Tarchalska-Krynska B, Opolski G, et al. Tryptase levels in patients after acute coronary syndromes: the potential new marker of an unstable plaque? *Clin Cardiol* 2003;26:366–72.
- [22] Cuculo A, Summaria F, Schiavino D, et al. Tryptase levels are elevated during spontaneous ischemic episodes in unstable angina but not after the ergonovine test in variant angina. *Cardiologia* 1998;43:189–93.
- [23] Van Haelst PL, Timmer JR, Crijns HJ, et al. No long-lasting or intermittent mast cell activation in acute coronary syndromes. *Int J Cardiol* 2001;78:75–80.
- [24] Patella V, Marino I, Arbustini E, et al. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. *Circulation* 1998;97:971.
- [25] Frangogiannis NG, Shimoni S, Chang SM, et al. Evidence for an active inflammatory process in the hibernating human myocardium. *Am J Pathol* 2002;160:1425–33.
- [26] Criqui MH, Lee ER, Hamburger RN, Klauber MR, Coughlin SS. IgE and cardiovascular disease. Results from a population-based study. *Am J Med* 1987;82:964–8.
- [27] Sedgwick JD, Holt PG, Turner KJ. Production of a histamine releasing lymphokine by antigen or mitogen stimulated human peripheral T cells. *Clin Exp Immunol* 1981;45:409–18.
- [28] Liu MC, Proud D, Lichtenstein LM, et al. Human lung macrophage-derived histamine-releasing activity is due to IgE-dependent factors. *J Immunol* 1986;136:2588–95.
- [29] Huang M, Pang X, Letourneau R, Boucher W, Theoharides TC. Acute stress induces cardiac mast cell activation and histamine release, effects that are increased in Apolipoprotein E knockout mice. *Cardiovasc Res* 2002;55:150–60.