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Metalloproteases 2 and 9, Lp-PLA$_2$ and Lipoprotein Profile in Coronary Patients

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Background and Aims. Many studies suggest that the different steps of the atherosclerotic process may be mediated by metalloproteases (MMPs). MMP-9 and MMP-2, which are highly expressed in the vulnerable regions of the atherosclerotic plaques, have been suggested to be causally involved in plaque rupture. In another manner linked with LDL, lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) hydrolyzes phospholipids generating proinflammatory and proatherogenic products. Our aim was to evaluate plasma activity of MMP-2 and 9, as well as Lp-PLA$_2$, in subjects with coronary artery stenosis in comparison with controls and to correlate these activities with lipoprotein profile and general biomarkers of inflammation.

Methods. Forty two subjects who had undergone coronary angiography were divided into two groups: patients with coronary vessels with at least 45% stenosis (CAD [coronary artery disease], $n = 24$) and patients without angiographically detectable coronary artery disease (controls, $n = 18$). Plasma activity of MMP-2 and MMP-9 was measured and correlated with markers of systemic inflammation (hs-CRP), subendothelial inflammation (Lp-PLA$_2$) and lipoprotein profile.

Results. Plasma activity of both MMPs was consistently higher in patients than in controls ($p < 0.01$). Pro-MMP-2 ($r = 0.34$, $p < 0.01$) and MMP-9 ($r = 0.51$, $p < 0.02$) activities correlated with apoprotein B. Pro-MMP-2 correlated with hs-CRP ($r = 0.47$, $p < 0.01$) and inversely with HDL cholesterol ($r = -0.35$, $p < 0.02$). No differences were observed in Lp-PLA$_2$ between patients and controls (15.2 ± 4.0 vs. 15.4 ± 4.5 μmol/mL/h, p = NS, respectively), and no correlation was observed with MMPs.

Conclusions. MMP activity was higher in CAD than in controls. The correlation observed between pro-MMP-2 and high-sensitive C-reactive protein (hs-CRP) may be due to specific systemic inflammatory processes. No correlation was observed between Lp-PLA$_2$ and MMPs.

Key Words: Metalloproteases, Plaque vulnerability, Lp-PLA$_2$, lipoproteins.

Introduction

Atherosclerosis is a multifactorial illness whose development and progression has been extensively studied (1–3). Many previous studies have suggested that the different steps of the atherosclerotic process may be mediated by metalloproteases. Matrix metalloproteases (MMPs) are a family of >20 zinc-dependent endopeptidases that collectively degrade most of the protein and proteoglycan-core-protein components of the extracellular matrix (ECM) (4). Increased expression and activity of these enzymes have been identified in various pathological processes such as general inflammation, tumor metastasis, myocardial injury and vascular remodelling (5). Thus, as

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MMPs play a significant role in vascular remodelling, they have been suspected to be partly responsible for the pathogenesis of cardiovascular disease. MMP-9 and MMP-2 are highly expressed in the vulnerable regions of the atherosclerotic plaques and, for this reason, have been suggested to be causally involved in plaque rupture (6). However, the hypothesis of the causal role of different MMPs in plaque rupture is controversial, given that they have been associated both with plaque instability (7) as well as with stability (8,9). Nevertheless, elevated plasma levels of different MMPs have been reported in patients with acute coronary syndrome and are associated with severe coronary stenosis and cardiovascular mortality.

Because high plasma concentration of low-density lipoprotein (LDL) is one of the principal risk factors for atherosclerosis, accumulation of this lipoprotein in the subendothelium has been extensively considered the primary event in atherosclerotic plaque formation. However, a substantial number of subjects with normal lipoprotein concentrations still develop atherosclerosis, thus supporting the presence of additional factors involved in this disease.

In association with LDL, the lipoprotein-associated phospholipase A_2 (Lp-PLA$_2$) has been described (10), an enzyme highly expressed in the damaged vessels that belongs to the A_2 phospholipase family that hydrolyzes phospholipids generating potent proinflammatory and proatherogenic products (11). Different studies also suggest that higher Lp-PLA$_2$ activity contributes to processes identified as pivotal to plaque vulnerability, including monocyte migration, proinflammatory effects of oxidized LDL and macrophage death (11,12). Recently, it has been demonstrated that Lp-PLA$_2$ induces pro-MMP-2 activation modulating the expression of tissue inhibitors of metalloproteases (TIMPs) by intracellular levels of cAMP (13). However, to our knowledge, no studies have evaluated the association between Lp-PLA$_2$ and MMPs activities in circulation.

It has been clearly established that vulnerable plaques are the underlying cause of most clinical coronary events. In some cases, a deep plaque injury cannot be identified despite a careful diagnostic search. Thus, it is important to identify predictors of instability in order to contribute to an early diagnosis and therapy and also to provide data to elucidate the mechanism underlying the process of instability.

Our aim was to evaluate plasma activity of MMP-2 and MMP-9, as well as Lp-PLA$_2$, in subjects with arterial stenosis in comparison with controls and to associate the activity of these enzymes with lipoprotein profile and biomarkers of general inflammation.

Materials and Methods

Studied Population

A total number of 42 Caucasian subjects were recruited at the Universidad Adventista del Plata, Paraná, Entre Rios, Argentina. These patients had undergone coronary angiography because of chest pain related to coronary heart disease, ischemic heart disease, myocardial infarction, and electrocardiography changes, as well as indication of valvular surgery. Patients presenting $>45\%$ stenosis were defined as cases (CAD [coronary artery disease], $n = 24$) warranting in all cases the presence of clinically significant lesions. Controls were selected among patients who had indication of coronary angiography previous to valvular surgery and who presented $<30\%$ stenosis ($n = 18$), without clinical significance (14). Patients presenting 30−45% stenosis were excluded. Each angiogram was performed with a Toshiba DS-TB (Toshiba, Tokyo, Japan) and subsequently reviewed independently by two experienced observers who were blinded to the clinical details. Patients taking cholesterol-lowering drugs were excluded, given that statins interfere in MMP synthesis (15,16). We evaluated the participants with regard to cigarette smoking, blood pressure and the presence of diabetes according to standard criteria. At the time of diagnosis, among those patients with hypertension, ten CAD and five controls were receiving antihypertensive treatment (beta blockers, calcium channel blockers or angiotensin receptor blockers). In addition, most of the diabetic patients were treated with oral hypoglycemic agents.

Written informed consent was obtained from each subject before admission, and the study protocol was approved by the Ethic Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires (Buenos Aires, Argentina).

Sample Collection

After an overnight fast, blood samples were obtained from peripheral vein puncture following a 15-min rest. Samples were collected in dry and chilled EDTA tubes for separating serum and plasma, respectively, within 1 h of the extraction. Serum samples were separated by centrifugation at 3,000 rpm during a period of 15 min at 4°C. For lipid and lipoprotein determinations, serum was kept at 4°C until its processing within 48 h. On the other hand, a serum aliquot was stored at −70°C for measurement of apoprotein B (apoB), Lp-PLA$_2$ and high-sensitive C-reactive protein (hs-CRP). For MMPs, a plasma aliquot was obtained because in serum samples MMPs are released from blood cells. In this case plasma was also kept at −70°C until processing.

Assay Procedure

Cholesterol and triglycerides (TG) were determined in a Hitachi 917 autoanalyzer by enzymatic methods (Roche Diagnostics, Mannheim, Germany). After selective precipitation methods, high-density lipoprotein (HDL) and LDL-cholesterol were determined (17,18). Serum lipid measurements were under good quality control with interassay coefficients of variation (CV) routinely $<3\%$. ApoB and hs-CRP were