*Biomarkers*, 2009; 14(2): 118–129

informa
healthcare

**RESEARCH ARTICLE**

# Potential implications of matrix metalloproteinase-9 in assessment and treatment of coronary artery disease

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**Abstract**

*Background*: Matrix metalloproteinase (MMP)-9, a member of the MMP superfamily is consistently implicated in the pathophysiology of atherosclerosis and plaque rupture, the most common mechanism responsible for acute coronary syndrome (ACS).

*Aim*: To summarize the role of MMP-9 in atherosclerosis and its potential implications in assessment and treatment of coronary artery disease (CAD).

*Methods*: We reviewed the PubMed database for relevant data regarding the role of MMP-9 in the pathophysiology of atherosclerosis. In the light of these data, we postulate potential implications of MMP-9 in the management and treatment of CAD.

*Results and conclusions*: Existing data strongly support the role of MMP-9 in plaque destabilization and rupture. Based on the current knowledge, MMP-9 can potentially serve as a diagnostic biomarker in ACS and a prognostic biomarker in ACS and chronic CAD patients. MMP-9 is reduced by therapies that are associated with favourable outcome in atherosclerosis and thus may serve as a surrogate biomarker of treatment efficacy. However, large morbidity and mortality trials are still required to confirm that MMP-9 reduction is associated with improved outcome independent of the traditional risk factors (i.e. low-density lipoprotein cholesterol). Given its role in plaque rupture, inhibition of MMP-9 may promote plaque stabilization and consequently reduce cardiovascular events. Yet, the efficacy and safety of MMPs inhibitors should be first studied in preclinical models of atherosclerosis.

**Keywords:** *Atherosclerosis; acute coronary syndrome; MMPs; MMP-9; MMP inhibitors*

## Introduction

The advancing knowledge of the pathophysiology of atherosclerosis introduced us to novel mediators that participate in the formation, development and complications of atherosclerosis. As current technology enables us to quantify plasma levels of many of these mediators, they could potentially serve as biomarkers reflecting disease burden or stability. Circulating biomarkers may be useful in the clinical assessment of patients with coronary artery disease (CAD) (i.e. diagnosis, risk stratification, prognostication) and for evaluation of novel therapies for atherosclerosis.

Tissue remodelling processes within the plaque, mainly regulated by matrix metalloproteinases (MMPs), play a major role in the pathophysiology of atherosclerosis and acute coronary syndrome (ACS). MMP-9, a member of the MMP superfamily has been consistently implicated in the pathophysiology of plaque rupture, the most common mechanism responsible for ACS. Subsequently, in this article we review the relevant data regarding the role of MMP-9 in atherosclerosis, and postulate potential implications of MMP-9 in the management and treatment of CAD.

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*(Revised 17 January 2009; accepted 21 January 2009)*

ISSN 1354-750X print/ISSN 1366-5804 online © 2009 Informa UK Ltd
DOI: 10.1080/13547500902765140

http://www.informapharmascience.com/bmk

## Background biology

Like most MMPs, MMP-9 is synthesized as pre-pro-enzyme containing a 'pre' domain that is removed during translation. The 'pro' domain which maintains enzyme latency is cleaved by activated MMPs, plasmin or other proteases, resulting in activation of the enzyme. MMP-9, also named gelatinase B or 92-kDa type collagenase is capable of degrading various matrix substrates including collagen types I, IV, and V, which constitute an important component of human atherosclerotic lesions (Rekhter 1999, Tayebjee et al. 2005a). MMP-9 is distinguished from other MMPs by an insertion of three cysteine-rich repeats in the catalytic domain that are required to bind and cleave extracellular matrix (Sternlicht & Werb 2001, Nagase et al. 2006). Under normal physiological conditions MMP-9 is secreted by osteoclasts of the bone growth plate, neutrophils, macrophages, trophoblast cells, hippocampal neurons and keratinocytes (Sternlicht & Werb 2001), whereas in human atherosclerotic plaques it is mainly expressed by macrophages and smooth muscle cells (Galis & Khatri 2002). MMPs are induced by several molecules including interleukin-1, tumour necrosis factor-α and platelet-derived growth factor, and inhibited by tissue inhibitors of metalloproteinases (TIMPs), transforming growth factor-β, heparin and corticosteroids (Dollery et al. 1995). *In vitro* activation of macrophage nuclear receptors liver X receptors (LXR) α and β have also been shown to inhibit the expression of MMP-9 in bone marrow-derived macrophages through inhibition of the nuclear factor κB pathway (Castrillo et al. 2003).

## The physiological role of matrix metalloproteinase-9

MMPs act as modulators of various physiological and pathological processes by cleavage of multiple peptidic substrates including chemoattractants, growth factors, adhesion molecules and structural extracellular matrix proteins (Table 1) (Sternlicht & Werb 2001). MMP-9 was first discovered as a neutrophil product over three decades ago. In neutrophils, it is synthesized by differentiating bone marrow granulocytes and released following neutrophil activation. Thereafter, it was also found to be produced by monocytes and lymphocytes (Hasty et al. 1990, Opdenakker et al. 2001). MMP-9 plays a role in stem cell mobilization, regulation of leukocytosis (Opdenakker et al. 2001, Starckx et al. 2002) transmigration of leukocytes (Delclaux et al. 1996), and angiogenesis.

Angiogenesis occurs during normal development and in many pathological conditions including tumour growth, inflammation and ischaemia. MMP-9, MMP-2, TIMP-2 and membrane type-1 MMP (MT1-MMP), have

**Table 1.** Matrix metalloproteinases (MMPs) associated with cardiovascular diseases in humans.

| MMP protein | Source | Family | Associated pathology |
|---|---|---|---|
| MMP-1 (Galis & Khatri 2002, Raffetto & Khalil 2008) | Smooth muscle cells, macrophages, endothelial cells | Collagenase | Atherosclerosis, hypertension, varicose veins |
| MMP-2 (Galis & Khatri 2002, Beaudeux et al. 2004, Raffetto & Khalil 2008) | Macrophages, fibroblasts, smooth muscle cells, keratinocytes, chondrocytes, endothelial cells and osteoblasts | Gelatinase | Atherosclerosis, hypertension, abdominal aortic and cerebral aneurysms, varicose veins |
| MMP-3 (Galis & Khatri 2002, Raffetto & Khalil 2008) | Macrophages, smooth muscle cells | Stromelysin | Atherosclerosis, abdominal aortic aneurysm, hypertension |
| MMP-7 (Galis & Khatri 2002, Raffetto & Khalil 2008) | Macrophages | Matrilysin | Atherosclerosis |
| MMP-8 (Galis & Khatri 2002, Turu et al. 2006, Wilson et al. 2006) | Macrophages, neutrophils, smooth muscle cells, endothelial cells | Collagenase | Atherosclerosis, abdominal aortic aneurysm |
| MMP-9 (Galis & Khatri 2002, Beaudeux et al. 2004, Raffetto & Khalil 2008) | Neutrophils, monocytes, macrophages, smooth muscle cells, osteoclasts, keratinocytes | Gelatinase | Atherosclerosis, hypertension, abdominal aortic aneurysm, varicose veins |
| MMP-10 (Rodriguez et al. 2008) | Endothelial cells | Stomelysin | Atherosclerosis, abdominal aortic aneurysm |
| MMP-11 (Schonbeck et al. 1999) | Macrophages, smooth muscle cells, endothelial cells | Stromelysin | Atherosclerosis |
| MMP-12 (Curci et al. 1998, Beaudeux et al. 2004, Raffetto & Khalil 2008) | Macrophages | Metalloelastase | Atherosclerosis, abdominal aortic aneurysm |
| MMP-13 (Mao et al. 1999, Galis & Khatri 2002, Raffetto & Khalil 2008) | Macrophages, smooth muscle cells | Collagenase | Atherosclerosis, abdominal aortic aneurysm, varicose veins |

all been shown to participate in the process of angiogenesis; they contribute to endothelial cell sprouting by degradation of extracellular matrix, adhesion molecules cleavage and modulation of growth factor release (Bendeck 2004, Tayebjee et al. 2005a). MMP-9 deletion was found to enhance neovascularization in post-myocardial infarction tissue and attenuate left ventricular (LV) dysfunction, suggesting that targeted strategies to inhibit MMP-9 early in post-myocardial infarction might improve angiogenesis, LV remodelling and LV function (Lindsey et al. 2006).

## MMP-9 and atherosclerosis formation

The association between MMP-9 expression and plaque formation was studied in apolipoprotein E (Apo E)-null mice model with conflicting results. According to Lutton et al. (2004), Apo E/MMP-9 double-knockout mice had 70% smaller sized plaques in the descending aorta with less collagen and macrophage content compared with Apo E knockout/MMP-9+/+ mice after 25 weeks of a cholesterol-rich diet, suggesting that MMP-9 deficiency protects from plaque development. Moreover, transplantation of Apo E knockout/MMP-9+/+ bone marrow into Apo E/MMP-9 double-knockout hosts restored the plaque size at the aortic origin to normal levels. MMP-9 was postulated by the authors to be involved in recruitment and migration of macrophages into the atherosclerotic plaques (Luttun et al. 2004).

In contrast, Johnson et al. (2005) demonstrated a significantly larger lesion area, increase in macrophage content and reduction in smooth muscle cell content in lesions from brachiocephalic arteries of Apo E/MMP-9 double-knockout mice compared with Apo E knockout/MMP-9+/+ mice after 8 weeks of a high-fat diet, proposing that MMP-9 deficiency promotes rather than impairs atherosclerosis progression (Johnson et al. 2005). The conflicting data could be explained by differences in timing of lesion assessment as Johnson et al. assessed the lesion size after 8 weeks of a high-fat diet, whereas Lutton et al. assessed the lesion size after 25 weeks of a cholesterol-rich diet, suggesting that the detrimental effect of MMP-9 occurs only at later stages of atherosclerosis. Of note, no differences in plaque size were noted by Lutton et al after 15 weeks of a cholesterol-rich diet, further supporting this hypothesis. Another possibility is that the unfavourable effect of MMP-9 is site specific, as Lutton et al. evaluated atherosclerotic lesions in the descending aorta, whereas Johnson et al. evaluated plaques in the brachiocephalic artery, which may not be comparable. Given the controversial findings, the precise role of MMP-9 in atherosclerosis development remains to be established.

## MMP-9 and plaque rupture

The role of MMP-9 in plaque rupture has been studied extensively in animal models of atherosclerosis and in humans, revealing a strong association between MMP-9 expression and ruptured plaque.

### *Animal studies*

Gough et al. have recently demonstrated that overexpression of activated MMP-9 by macrophages induced significant plaque disruption in advanced atherosclerotic lesions of Apo E knockout mice, revealing that enhanced macrophage proteolytic activity with MMP-9 can induce acute plaque disruption and highlighting MMP-9 as a potential therapeutic target for stabilizing rupture-prone plaques (Gough et al. 2006). According to de Nooijer et al. the unfavourable effect of MMP-9 overexpression on plaque stability appears to be more prominent in advanced atherosclerotic plaques. While intermediate lesions revealed outward remodelling and increased prevalence of vulnerable plaque morphology, advanced plaques displayed more significant features of vulnerable plaque with a high incidence of intraplaque haemorrhage (de Nooijer et al. 2006). A novel fluorescence imaging technique has utilized a near-infrared fluorescence (NIRF) probe to detect enzymatic activity of MMP-9 in Apo E knockout mice. This novel technique enabled visualization of atherosclerotic lesions in the aortas of Apo E knockout mice. Subsequent treatment with gelatinase inhibitor markedly reduced the NIRF signal demonstrating the specificity of the signal to gelatinase activity (Deguchi et al. 2006). In the future, such novel modalities might be used for detection of unstable plaques as a target for intravascular interventions.

### *Human studies*

Increased expression of MMP-9 has been consistently found in atherosclerotic plaques of patients experiencing ACS or ischaemic stroke (Brown et al. 1995, Loftus et al. 2000, Stintzing et al. 2005). Galis et al. were among the first to show that MMP-9 is expressed in human atherosclerotic lesions, mainly in the plaque shoulders and regions of foam cell accumulation that are prone to rupture, whereas non-atherosclerotic arteries stained weakly or not at all for MMP-9. Increased gelatinolytic activity attributed to MMP-9 and MMP-2 was also demonstrated in frozen sections of atherosclerotic plaques as opposed to normal arteries (Galis et al. 1994). Similar findings were reported by Stintzing et al. who demonstrated higher expression of MMP-9, particularly in the shoulder and cap regions of complicated carotid plaques obtained from post-ischaemic stroke patients, compared with non-atherosclerotic arteries (Stintzing

et al. 2005), supporting the correlation between MMP-9 expression within plaque regions that are prone to rupture and thromboembolic events. Brown et al. showed that active synthesis of MMP-9 occurred mainly in patients with unstable angina as opposed to stable angina patients, suggesting a pathogenic role for MMP-9 in ACS. Although 83% of coronary atherectomy specimens from patients with unstable or stable angina were positive for MMP-9, intracellular localization of MMP-9, indicating of active synthesis, was documented in all positively stained specimens of unstable angina patients but only in 30% of positively stained specimens from stable angina patients (Brown et al. 1995).

Increased expression of MMP-9 was demonstrated in carotid plaques of patients who experienced a recent cerebrovascular ischaemic event compared with patients with a less recent cerebrovascular ischaemic event or asymptomatic patients. Moreover, the concentration of MMP-9 was significantly higher in plaques with histological findings of plaque instability and rupture ($p < 0.03$), indicating the role of MMP-9 in the pathophysiology plaque rupture (Loftus et al. 2000).

## MMP-9 and TIMPs

The TIMPs represent a family of four secreted proteins (TIMPs 1–4) that reversibly inhibit MMPs. TIMPs are capable of inhibiting the activities of MMPs by binding in a 1:1 stoichiometry, and play a key role in maintaining the balance between extracellular matrix (ECM) deposition and degradation (Sternlicht & Werb 2001). Imbalance of the MMP/TIMP ratio is in favour of ECM degradation, and hence it is conceivable that it can serve as a biomarker for atherosclerosis progression. Experimental studies using knockout mice models demonstrated the importance of MMP/TIMP balance in atherosclerotic lesion progression. Apo E knockout mice overexpressing TIMP-1 exhibited less advanced atherosclerosis than Apo E knockout control mice. Similarly, deficiency of the MMP-9 gene resulted in decreased intimal thickening and migration of smooth muscle cells and TIMP-1/Apo E double-knockout mice exhibited an increase in vascular medial degradation after 10 weeks of a high-fat diet, that was associated with larger atherosclerotic lesions (Beaudeux et al. 2004). Accordingly, in humans, the ratio of MMP-9/TIMP-1 was significantly higher in ACS patients compared with the control group and was found to be an independent predictor of ACS and complex plaques (Cheng et al. 2008).

## Circulating MMP-9 and CAD

In accordance with the histological findings, plasma levels of MMP-9 are increased in clinical conditions that are associated with plaque rupture, including unstable angina and acute myocardial infarction (AMI), indicating its potential role as a biomarker for clinical assessment of ACS patients.

## Acute coronary syndrome

Serial measurements of MMP-9 during the first week of ACS revealed different time-course patterns of MMP-9 elevation. Two studies enrolling patients with AMI demonstrated peak levels on days 0 and 3 of admission and a nadir on day 1 (Squire et al. 2004, Tziakas et al. 2004), whereas a third study demonstrated only one peak on day 0. The differences may be related to the thrombolytic therapy that was given to many of the patients in the first two studies, whereas none of the patients in the third study received thrombolytic therapy, as thrombolytic therapy was reported to increase MMP activity (Kelly et al. 2006). MMP-9 levels are also increased in unstable angina. In one study the level was highest on day 0 and declined to a normal range on day 7 whereas, in another study MMP-9 was constantly elevated during the first 30 days after admission (Kai et al. 1998, Tziakas et al. 2004). Several small cross-sectional studies demonstrated a 3.3-fold increase in MMP-9 among ACS patients compared with healthy subjects (Kai et al. 1998, Zeng et al. 2005) and a 1.5–2.6-fold increase compared with stable angina patients (Kai et al. 1998, Manginas et al. 2005, Zeng et al. 2005) indicating the potential value of MMP-9 as a diagnostic biomarker for ACS. Yet, further prospective studies are still required to determine its specificity and sensitivity, and its advantage over the traditional biomarkers of myocardial necrosis, creatine phosphokinase (CPK) and troponin.

The precise source of plasma MMP-9 has not been established although based on existing data it is conceivable that it is released from the ruptured plaque itself.

Funayama et al. measured MMP-9 in selective cardiac catheterization of patients with AMI demonstrating higher levels in the infarct-related artery than in the aorta indicating regional release of MMP-9 from the culprit plaque (Funayama et al. 2004). Nevertheless, it cannot be excluded that MMP-9 was derived from ischaemic ventricular tissue rather than the ruptured plaque itself (Thompson & Squire 2002).

MMP-9 was also found to be an independent predictor of plaque rupture assessed by intravascular ultrasound of the culprit lesion in patients presenting with AMI or UA, even after adjustment for the traditional risk factors, C-reactive protein (CRP) and troponin. Patients with ACS and plaque rupture had 1.4–2.0-fold increases in MMP-9 compared with ACS patients without plaque rupture, supporting the association between plasma MMP-9 and plaque rupture (Fukuda et al. 2006).

One may argue that a single atherosclerotic plaque is insufficient to release the amount of plasma MMP-9 that is seen in patients with ACS. Yet, several studies have shown that ACS is associated with diffuse destabilization of atherosclerotic plaques rather than a single ruptured plaque. Goldstein et al. analyzed angiograms of patients presenting with AMI, revealing multiple complex plaques in approximately 40% of the patients (Goldstein et al. 2000). Rioufol et al. (2002) who evaluated all three coronary arteries by intravascular ultrasound showed more than one ruptured plaque in approximately 80% of ACS patients and a recent histopathological study demonstrated diffuse and active inflammation in both vulnerable and stable plaques of patients dying of AMI compared with plaques of patients dying of chronic stable angina or non-cardiac causes (Mauriello et al. 2005).

To date, only one prospective study has evaluated the predictive role of MMP-9 in CAD patients. MMP-9, measured in a large prospective study of the AtheroGene group, was found to be an independent predictor of cardiovascular (CV) death in the entire cohort of patients consisting of 795 patients with stable angina and 332 patients with unstable angina, even after adjustment for multiple variables including traditional risk factors and therapies applied. The authors suggested that MMP-9 could serve as a novel biomarker for prognostication of CAD patients (Blankenberg et al. 2003).

## Stable angina

In contrast to ACS patients, most studies enrolling stable CAD patients reported comparable levels of MMP-9 in stable angina patients and controls, indicating that circulating MMP-9 is not useful for diagnosis of stable CAD patients. MMP-9 measured by enzyme-linked immunosorbent assay (ELISA) was similar in patients with stable effort angina and healthy controls (Kai et al. 1998) and was increased by only 1.1-fold in patients with stable CAD compared with age- and gender-matched healthy controls ($p = 0.0099$) with a significant overlap between the two groups (Tayebjee et al. 2005b).

In contrast, a single study that assessed MMP-9 activity by quantitative gelatin zymography found a 2.1-fold increase in active MMP-9 in stable angina patients compared with non-CAD subjects (Zeng et al. 2005), suggesting that measurement of MMP-9 activity may be more sensitive for differentiation of stable CAD patients than absolute MMP-9 level measured by ELISA assay.

Unexpectedly, although cross-sectional studies were mostly negative, plasma MMP-9 was found to predict CV death in a subanalysis of 791 stable CAD patients enrolled in the prospective AtheroGene study. Baseline MMP-9 was found to be an independent predictor of CV death in stable CAD patients, after adjustment for risk factors, therapies and ejection fraction (hazard risk ratio of patients in the 4th vs 1st quartile 2.88, 95% confidence interval (CI) 1.19–6.95, $p = 0.02$) (Blankenberg et al. 2003). Similarly, MMP-9 was found to predict long-term adverse outcome among patients with >50% carotid stenosis. MMP-9 above versus below the median had a hazard ratio of 1.9 for ipsilateral stroke or CV death (95% CI 1.1–3.5), supporting its role as a predictive biomarker in the vast spectrum of atherosclerosis (Eldrup et al. 2006).

## Traditional risk factors and MMP-9

MMP-9 is associated with several of the traditional risk factors for atherosclerosis including hypertension, smoking, diabetes and dyslipidaemia.

### *Hypertension*

Hypertension is a multifactorial disorder involving various organs including the kidney, central nervous system, endocrine system and vasculature. It is characterized by structural changes in the arterial wall caused by increased deposition of ECM components, and variations in ECM architecture. Thus, it is conceivable that hypertension is associated with alterations in MMP and TIMP activity.

Increased pretreatment plasma MMP-9 was found in 96 hypertensive patients versus 45 age- and sex-matched healthy controls enrolled in a substudy of the Anglo Scandinavian Cardiac Outcome Trial (ASCOT), assessing patients with uncontrolled hypertension before and after cardiovascular risk management (Tayebjee et al. 2004a). MMP-9 was positively correlated with the Framingham cardiovascular risk score (Tayebjee et al. 2004a). In a second study, MMP-9 was increased in 74 hypertensive patients with diastolic dysfunction and increased LV mass compared with 34 control subjects. MMP-9 was not correlated with echocardiographic parameters of diastolic dysfunction, implying that it is unrelated to myocardial pressure overload (Tayebjee et al. 2004b). In contrast, two other studies reported of lower MMP-9 among hypertensive patients versus control subjects, suggesting that it may reflect abnormal ECM metabolism (Li-Saw-Hee et al. 2000, Zervoudaki et al. 2004).

Given the controversy in the literature, Derosa et al. have analyzed MMP-9 in 44 subjects with hypertension and 44 control subjects. An approximately 10-fold increase in MMP-9 level was seen in hypertensive patients compared with normotensive controls indicating greater vascular/cardiac matrix turnover in hypertension ($p < 0.0001$). The authors could not find a clear explanation for the variable results in the literature (Derosa et al. 2006). Our impression is that additional

variables such as duration of hypertension prior to enrolment and renal function, which were not assessed in these studies, might elucidate the discrepancy.

### *Smoking*

Only a few studies have assessed the correlation between MMP-9 and smoking. Nakamura et al. evaluated the effect of cigarette smoking on plasma MMP-9 concentrations in healthy smoking subjects versus non-smoking subjects; MMP-9 was found to be higher by 1.5-fold in the smoking group and was positively correlated with smoking duration (Nakamura et al. 1998). More recently, increased MMP-9 has been demonstrated in smoking subjects participating in the Framingham Heart Study even after adjustment for age and sex (Sundstrom et al. 2004). However, the precise mechanism of MMP-9 elevation among smokers has not been established yet.

### *Diabetes*

The contribution of hyperglycaemia to complications of atherosclerosis has been studied in macrophage cell lines by measurement of MMP expression and activity in response to high glucose exposure. MMP-9 activity and mRNA expression were found to increase in response to a hyperglycaemic state, suggesting a mechanism by which the hyperglycaemic state adversely contributes to cardiovascular complications (Death et al. 2003). In accordance, MMP-9 level has been consistently shown to increase in diabetic patients. In the Framingham Heart Study enrolling patients free of heart failure and previous myocardial infarction, MMP-9 was significantly higher in diabetic versus non-diabetic patients even after adjustment for age and sex (Sundstrom et al. 2004). Marx et al. (2003) assessed the correlation between MMP-9 and CAD in patients with and without diabetes, demonstrating significantly higher MMP-9 levels in diabetic patients with CAD compared with non-diabetic patients with CAD. The authors proposed that this finding might reflect the presence of more unstable lesions among diabetics than non-diabetics (Marx et al. 2003). Recently, a higher MMP-9 level was found in type 1 diabetic patients versus non-diabetic subjects, and in diabetic patients with retinopathy versus diabetic patients without retinopathy (Jacqueminet et al. 2006). Of note, thus far MMP-9 has not been found to correlate with the duration of diabetes (Maxwell et al. 2001, Marx et al. 2003, Jacqueminet et al. 2006).

### *Dyslipidaemia*

Dyslipidaemia has been correlated with increased MMP-9 levels even in the absence of known atherosclerotic disease; however, the mechanism has not been clarified so far. Beaudeux et al. reported of increased MMP-9 levels among asymptomatic hyperlipidaemic patients defined as total cholesterol >5.2 $mmol^{-1}$ and/or triglycerides >1.7 $mmol^{-1}$ compared with age- and gender-matched non-hyperlipidaemic subjects (Beaudeux et al. 2003). Moreover, among stable CAD patients with angiographically confirmed disease, MMP-9 was positively correlated with low-density lipoprotein (LDL) cholesterol and negatively correlated with high-density lipoprotein (HDL) cholesterol levels (Noji et al. 2001). Negative correlation between HDL cholesterol was also demonstrated in patients with hypertension participating in the ASCOT trial (Tayebjee et al. 2004a).

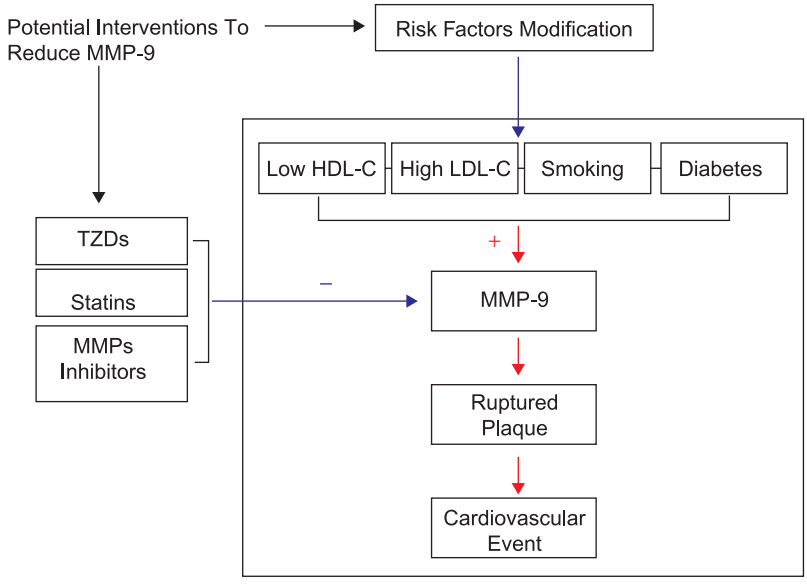


**Figure 1.** Postulated interactions between the traditional risk factors, matrix metalloproteinase (MMP)-9, cardiovascular events and therapeutic interventions. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TZDs, thiazolidinediones.

In conclusion, existing data suggest that the traditional risk factors including diabetes, smoking and dyslipidaemia are associated with increased MMP-9 levels, even among patients without evidence of atherosclerosis. MMP-9 may thus serve as a potential link between the traditional risk factors and atherosclerosis development and complications (Figure 1).

## Therapies and MMP-9

As MMP-9 is involved in plaque destabilization and rupture, therapies directed at reducing MMP-9 expression or inhibition of MMP-9 activity may promote plaque stabilization and consequently reduce CV events.

### *HMG Co-A reductase inhibitors*

HMG Co-A reductase inhibitors (statins) are suggested to exert many beneficial effects beyond their LDL-cholesterol-lowering effect, including attenuation of the inflammatory response (Ridker et al. 2005), improving endothelial function (Laufs & Liao 2000) and reducing thrombogenicity (Almuti et al. 2006). The effect of statins on MMP-9 was assessed in several trials, demonstrating a dose-dependent reduction in MMP-9 expression and activity *in vitro* and *in vivo*, suggesting a favourable tissue remodelling effect. Fluvastatin reduced MMP-9 activity and expression in cultured human macrophages (Bellosta et al. 1998), cerivastatin reduced MMP-9 secretion from rabbit foam cells, and several other statins including lovastatin, simvastatin and cerivastatin inhibited inducible MMP-9 secretion from human smooth muscle cells (Luan et al. 2003).

Statin treatment has also been shown to reduce MMP-9 expression within the atherosclerotic plaque itself. MMP-9 expression and activity in endarterectomy specimens extracted from asymptomatic type 2 diabetic patients treated by simvastatin and diet for 4 months were significantly lower than those of patients treated with diet alone (expression: 22% vs 7%, activity: 2734 vs 565 DU, respectively, $p < 0.0001$) (Cuccurullo et al. 2006).

In accordance, statin treatment was strongly associated with lower plasma MMP-9 concentration among 1127 patients with stable and unstable angina. Approximately 23% of patients in the 4th quartile of MMP-9 were treated with statins compared with 40% of patients in the 1st quartile of MMP-9 ($p < 0.0001$) (Blankenberg et al. 2003). Prospective studies also revealed reduction in MMP-9 levels with statin therapy. Twelve weeks of fluvastatin treatment was shown to reduce MMP-9 by 32% ($p = 0.023$) among subjects with hypercholesterolaemia, independent of its LDL-cholesterol-lowering effect (Leu et al. 2005) and 4 weeks of therapy with atorvastatin and conventional therapy in ACS patients reduced plasma MMP-9 levels to a greater extent than conventional therapy alone (57% vs 33% reduction, respectively, $p < 0.05$) (Xu et al. 2004). Similarly, 12 weeks of therapy with atorvastatin or rosuvastatin were shown to reduce MMP-9 levels by 28% and 31%, respectively, among 69 consecutive patients with primary hypercholesterolaemia ($p < 0.05$ compared with baseline) (Qu et al. 2008).

To summarize, statins therapy reduces MMP-9 expression *in vitro* and *in vivo*, including in macrophage cultures, atherosclerotic lesions and within the plasma, suggesting a novel non-LDL-lowering therapeutic effect for statins.

### *Thiazolidinediones*

Thiazolidinediones (TZDs) are agonists of the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ), which regulates transcription of target genes involved in glucose homeostasis, fatty acid metabolism and inflammation. TZDs improve insulin resistance and are widely used for the treatment of type 2 diabetic patients (Staels & Fruchart 2005).

TZDs have been shown to reduce CV events among diabetic patients. Pioglitazone treatment in the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) resulted in significant reduction in the main secondary endpoint of the study, which consisted of all-cause mortality, non-fatal myocardial infarction and stroke among diabetic patients with established cardiovascular disease (Dormandy et al. 2005).

TZDs have other favourable effects beyond glycaemic control including improved thrombogenicity (Haffner et al. 2002), endothelial function (Calnek et al. 2003) and tissue remodelling (Blaschke et al. 2006). *In vitro*, troglitazone (Marx et al. 1998) and rosiglitazone (Patel et al. 2002) were found to inhibit MMP-9 expression in monocyte cultures in a concentration-dependent manner.

*In vivo*, assessment of rosiglitazone effect on MMP-9 in type 2 diabetic patients demonstrated a significant and dose-dependent reduction in MMP-9 after 26 weeks compared with baseline and placebo, whereas no change was observed in the placebo group (Haffner et al. 2002). In accordance, Marx et al. demonstrated a significant reduction in serum MMP-9 with rosiglitazone in type 2 diabetes patients and angiographically proven CAD after only 2 weeks of treatment, which remained at 12 weeks and was not observed in the placebo group. The authors postulated that this finding supports the pleiotropic effect of TZDs (Marx et al. 2003). A significant reduction in carotid intima-media thickness and several inflammatory biomarkers including high-sensitivity CRP, monocyte chemoattractant protein-1 and MMP-9 was found in type 2 diabetic patients treated with pioglitazone versus glimepiride for 26 weeks. As final HbA1c was not different between the two groups, the authors concluded that the favourable effect of pioglitazone was independent of glycaemic control (Pfutzner et al. 2005).

### *Matrix metalloproteinase inhibitors*

Based on the role of MMPs in the pathogenesis of atherosclerosis, inhibition of MMPs could potentially serve as a novel mechanism for the treatment of atherosclerosis. As MMPs are modulators of many physiological activities, selective inhibition of MMP-9 may improve plaque stabilization with less toxicity than non-selective inhibition of MMPs, whereas non-selective inhibition may be more effective, as other MMPs are also implicated in the pathogenesis of atherosclerosis (Sundstrom & Vasan 2006).

To date, MMP inhibitors (MMPIs) have been investigated as an anti-cancer therapeutic modality in patients with advanced disease. The rationale for development of MMPIs for cancer is based on the ability of MMPs to degrade the basement membrane and the ECM, thereby facilitating the invasion of malignant cells through connective tissues, resulting in tumour growth and metastasis.

Currently, inhibition of MMPs activity is the most commonly developed mechanism for MMPIs, although other approaches have been studied including inhibition of signal transduction responsible for MMP expression, or inhibition of MMP expression by specific antisense oligonucleotides. The latter approach may be favourable as it is more specific and potentially might have fewer side-effects (Hidalgo & Eckhardt 2001). MMPIs are categorized into three pharmacological groups.

#### Peptidomimetic MMPIs

Peptidomimetic MMPIs are pseudopeptide derivatives that mimic the structure of collagen at the binding site of MMPs. The inhibition is directed at the active site of MMPs in a reversible fashion. The first peptidomimetic MMPIs evaluated in cancer patients was batimastat, a non-selective MMPI directed against MMP–1, –2, –3, –7 and –9. Batimastat was mainly limited by its non-oral bioavailability (Hidalgo & Eckhardt 2001). The newer compound, marimastat has an oral bioavailability with similar inhibitory effects to batimastat. It displays favourable pharmacological properties, with a linear pharmacological behaviour and good absorption. However, marimastat is associated with dose-limiting inflammatory polyarthritis (Pavlaki & Zucker 2003). The newer peptidomimetic MMPI, prinomastat, which acts against MMP–2, –9, –13 and –14 is still associated with significant musculoskeletal toxicity that resolves with dose reduction (Scatena 2000).

#### Non-peptidic MMPIs

The lack of specificity led to the development of non-peptidic MMPIs that are more selective for MMP subtypes. Several non-peptidic MMPIs have been developed so far with various levels of selectivity. One of the more selective non-peptidic compound is BMS-275291, an orally bioavailable MMPI directed towards MMP-2 and -9 (Hidalgo & Eckhardt 2001). BMS-275291 is currently being studied in phase II and III trials in different cancer types. Although various degrees of side-effects including musculoskeletal toxicity, rash, flu-like symptoms and hypersensitivity reactions were reported when administered in combinations with other chemotherapies (Douillard et al. 2004, Miller et al. 2004, Leighl et al. 2005) it was well tolerated among patients when administered as a single agent, without evidence of dose-limiting musculoskeletal toxicity (Lara et al. 2006).

These data suggest that administration of more selective MMPIs may be favourable than peptidomimetic MMPIs. Caution should be applied towards their potential anti-angiogenic effect, which is beneficial in patients with metastatic cancer, but may be harmful in ischaemic heart disease. Selective inhibition of MMP-9 may not be associated with an anti-angiogenic effect as MMP-2 and membrane type-1 MMP (MT1-MMP) are the major MMPs that have been associated with angiogenesis so far (Zucker et al. 2000). In addition, MMP-9 deletion in a post-myocardial infarction mice model was found to stimulate rather than impair neovascularization in the remodelling tissue. The efficacy and safety of non-peptidic MMPIs must first be assessed in preclinical models of atherosclerosis.

#### Tetracycline derivatives

Tetracyclines consist of the traditional tetracycline antibiotics and the newer tetracycline analogues without antimicrobial activity. Tetracycline inhibits both activity and production of MMPs and hence may be a more potent inhibitor than the other MMPIs (Hidalgo & Eckhardt 2001).

The traditional tetracycline antibiotic doxycycline has been shown to inhibit the secretion of MMP-9 *in vitro* (Fife & Sledge 1995, Hanemaaijer et al. 1998). To date, only a few retrospective studies have evaluated the association between tetracycline treatment and CV events, in relation to *Chlamydia pneumoniae* infection, with conflicting results. (Jackson et al. 2000, Kardara et al. 2006).

The newer generation of chemically modified tetracyclines (CMTs) constitutes a group of more than 10 analogues with various specificity and potency. One of the most studied CMTs so far is CMT-3 (COL-3 or metastat) which inhibits the activity, activation and production of MMP-2 and -9 (Hidalgo & Eckhardt 2001). COL-3 has been studied in phase I and II trials in patients with Kaposi's sarcoma during a median term of 17–27 weeks demonstrating significant reduction in MMP-2 and MMP-9 levels. The most commonly reported adverse events were dosing-related photosensitivity and rash which were also the most common causes for dose reduction or termination of treatment.

**Bisphosphonates**

Bisphosphonates are stable analogues of pyrophosphate. They are widely used for the treatment of osteoporosis due to their ability to inhibit osteoclasts and bone resorption and are also implicated in the treatment of cancer, in part because of their ability to inhibit MMPs (Neville-Webbe et al. 2002).

Bisphosphonates were shown to inhibit MMP activity *in vitro* in a non-selective and dose-dependent manner (Neville-Webbe et al. 2002). Clodronate, pamidronate and alendronate have been all found to inhibit MMP-9 activity; however, they also inhibit several other MMPs including MMP–1, –2 and –8 (clodronate), MMP–1, –2, –3 and –8 (pamidronate) and MMP–3, –8, –12 and –13 (alendronate) (Teronen et al. 1999).

The role of bisphosphonates in the treatment of atherosclerosis has been studied in animal models demonstrating inhibition of atherosclerosis without alteration of the serum lipid profile (Kramsch & Chan 1978, Kramsch et al. 1981). However, none of these studies evaluated the association between atherosclerosis inhibition and MMP-9 expression.

## Summary

Current data strongly support the role of MMP-9 in the pathophysiology of atherosclerosis, and plaque rupture, the most common mechanism of ACS. Based on the existing data, MMP-9 may potentially serve as a diagnostic biomarker for patients presenting with acute chest pain although further prospective studies are still required to assess the precise time course of MMP-9 elevation during the first hours of symptoms and whether or not MMP-9 elevation is independent of creatine kinase and troponin elevation. Currently, data regarding its predictive value in CAD patients is sparse but appear promising. Larger prospective studies are still required to establish its role in risk stratification of CAD patients.

Several treatments that are known to improve outcome in CAD, including statins, have been found to reduce MMP-9 levels. As such, MMP-9, could potentially serve as a surrogate biomarker to monitor novel therapies for atherosclerosis. Nevertheless, the use of MMP-9 as a surrogate biomarker necessitates large morbidity and mortality trials in order to demonstrate that the reduction in MMP-9 levels is associated with improved outcome, independent of the traditional risk factors of atherosclerosis such as LDL-cholesterol.

Lastly, based on the role of MMP-9 in plaque destabilization and rupture, MMP-9 inhibition may serve as a novel therapy for atherosclerosis. Inhibition of MMP-9 can enhance plaque stabilization and subsequently reduce CV events. Yet, the efficacy and safety of MMPIs must be first evaluated in preclinical models of atherosclerosis.

## Acknowledgements

***Declaration of interest*:** Tu T Nguyen, Robert Wolk, Robert J Aiello, Steven G Terra, and David A Fryburg are all employees and shareholders of Pfizer, Inc. This paper was prepared while Yuval Konstantino was supported by the Israel Heart Society-Pfizer postdoctoral fellowship in cardiovascular drug development.

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