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Monocyte matrix metalloproteinase production in Type 2 diabetes and controls – a cross sectional study

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Abstract

Background: Coronary plaque rupture may result from localised over expression of matrix metalloproteinases (MMPs) within the plaque by infiltrating monocyte – macrophages. As MMP expression can be promoted by the modified lipoproteins, oxidative stress and hyperglycaemia that characterises Type 2 diabetes, we hypothesised that peripheral monocytes in these patients, exposed to these factors in vivo, would demonstrate increased MMP production compared to controls.

Methods: We examined peripheral venous monocyte expression of MMP and tissue inhibitor of metalloproteinase-1 (TIMP-1) in 18 controls and 22 subjects with Type 2 diabetes and no previous cardiovascular complications.

Results: No significant difference in MMP-1, 3 or 9 or TIMP-1 production was observed between control and diabetes groups.

Conclusions: Monocyte MMP-1, 3, and 9, and TIMP-1, production are not abnormal in Type 2 diabetes. This data cannot be extrapolated to monocyte – macrophage behaviour in the vessel wall, but it does suggest MMP and TIMP-1 expression prior to monocyte infiltration and transformation are not abnormal in Type 2 diabetes.

Background

The risk of an acute coronary syndrome is dependent on the stability of the atheromatous coronary plaque, and plaque stability and rupture may be mediated by focal over-expression in the plaque of matrix metalloproteinases or MMPs [1]. The MMPs are a family of proteinases capable of degrading all extracellular matrix (ECM) components and whose activity is tightly controlled by endogenous inhibitors known as tissue inhibitors of metalloproteinases or TIMPs [2]. The expression of a number of MMPs are up-regulated in atherosclerotic plaques ; in particular MMPs-1, -3, -7 and -9 have been localised to regions of macrophage – derived foam cell accumulation in the vulnerable plaque shoulders where rupture often occurs [3,4]. Type 2 diabetes is associated with a substantially increased risk of an acute coronary event [5], and is characterised by abnormalities in a number of factors that could modulate the expression of monocyte-derived

MMPs such as plasma tumour necrosis factor-alpha (TNF-α) [6,7], modified lipoproteins [8,9], hyperglycaemia and changes in oxidative balance [10]. Atherosclerotic plaque foam cells are mainly derived from infiltrating peripheral monocytes that have differentiated into macrophages during extravasation and taken up lipoproteins via newly expressed scavenger receptors such as CD36 but there is no available adequate data on monocyte or macrophage MMP or TIMP expression in Type 2 diabetes [11]. We hypothesised that monocytes derived from subjects with Type 2 diabetes might demonstrate altered MMP production due to exposure to such factors *in vivo*.

Methods

Subjects

Subjects with Type 2 diabetes and without known coronary artery disease (n = 22) were recruited from the Elsie Bertram Diabetes Centre, Norwich if they had Type 2 diabetes based on the American Diabetes Association criteria [12] and no clinical or electrocardiographic evidence of ischaemic heart disease. Coronary angiography or stress testing were not undertaken as part of this protocol, but patients with a history of previous confirmed myocardial infarction, or 12 – lead or exercise electrocardiogram evidence of myocardial infarction or ischaemic heart disease were excluded. Controls without Type 2 diabetes (n = 18) had no clinical history or electrocardiographic evidence of vascular disease, and all had a fasting plasma glucose below 6.1 mmol/l [12] and no patient with impaired fasting glucose was included in the control or diabetes group. No patient or control was taking aspirin or lipid lowering therapy. No subjects with Type 2 diabetes had macroproteinuria or microalbuminuria as determined by an elevated early morning albumin : creatinine ratio, and active smokers or subjects taking insulin were excluded. Thirteen of the 22 patients with Type 2 diabetes were taking a sulphonylurea (either alone in combination), of whom 7 were taking gliclazide. No patient was taking rosiglitazone or pioglitazone.

Primary human monocyte isolation

Monocytes were purified from plasma by density gradient centrifugation after collection of peripheral blood into EDTA-containing tubes as previously described [13]. Monocyte purity was greater than 85% and cell viability (assessed by trypan blue exclusion) was normally greater than 95% [13].

Monocyte culture

The monocytes were resuspended in RPMI 1640 medium (Gibco) containing 25 mmol/L glucose, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, non-essential amino acids, penicillin, streptomycin. Polymixin B was also included in the medium to avoid possible stimulation by endotoxin. The cells were cultured in duplicate for 48 hours, 37°C, 5% CO_2, at a density of 250 000/well of 24-well tissue culture dish (Costar) in a total volume of 0.5 ml. After 48 hours cell viability was assessed by measuring lactate dehydrogenase release using a cytotoxicity detection kit (Boehringer-Mannheim). Cell viability at the end of the culture period was normally 70%. Conditioned media was collected, centrifuged to remove cell debris and frozen at -20°C.

MMP analysis

Total MMP-3, total TIMP-1, pro-and complexed MMP-9 levels in monocyte conditioned media were measured using the Biotrak human ELISA systems (Amersham Pharmacia Biotech UK) according to the manufacturers instructions. Total MMP-1 was assessed using an in-house double antibody sandwich ELISA employing the monoclonal capture antibody RRU-CL1 and a biotinylated polyclonal anticollagenase IgG [14]. To detect the conversion of pro-to active MMP-9 gelatin zymography was carried out as previously described [15]. Conditioned media diluted in non-reducing sample buffer was subjected to SDS-PAGE on 10% (w/v) polyacrylamide gels incorporating 0.1% (w/v) gelatin. After electrophoresis the gel was washed in 2 × 15 minute washes of 2.5% (v/v) Triton X-100 followed by overnight incubation at room temperature in 50 mM Tris, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$. The gel was stained with 0.25% (w/v) Coomassie blue R-250, 50% (v/v) methanol, 10% (v/v) acetic acid, and destained in 30% (v/v) methanol, 1% (v/v) acetic acid. Enzyme activity appeared as cleared bands where the substrate had been degraded.

Statistical analysis

Data for MMP-1, MMP-3, MMP-9 and TIMP-1 were not normally distributed and are expressed as a median with interquartile range. Differences between groups were analysed by two-tailed Mann-Whitney U tests. Relationships between variables were assessed using simple linear regression. < 0.05 was taken as significant.

Although no available data is available to allow definitive power calculations, sample sizes were selected to offer more than 80 % power at the 5 % level to detect one standard deviation difference between group means assuming normal and equal variance between groups.

Results

Clinical features (Table 1)

Clinical features of the groups are shown in Table 1.

Basal monocyte MMP expression (Table 1)

MMP-9 and TIMP-1 were abundantly expressed in the cultured primary human monocytes when compared to MMP-1, and MMP-3, production was negligible (median 0.0 ng/ml). Using gelatin zymography it was shown that

Table 1: Clinical features, monocyte matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinases (TIMP) expression in controls and in Type 2 diabetes patients

	Control	DM CVD-
n	18	22
Age (yrs)	51.7 (8)	56.0 (8) *
Male : Female	14:4	16:6
Diabetes duration (yrs)	N/A	6.5 (5.9)
BMI (kg/m2)	26.6 (6.0)	27.4 (7.2)
HbA1c (%)	5.1 (0.4)	7.0 (1.4) **
Fasting plasma glucose (mmol/l)	4.7 (0.7)	8.6 (2.9) **
Treatment		
Aspirin	0	0
Statin	0	0
Diet alone	-	6
Sulphonylurea alone	-	8
Metformin alone	-	3
Combination	-	5
MMP-1 (ng/ml)	5.5 (5)	6.4 (5.9)
MMP-3 (ng/ml)	0.0 (1.2)	0.0 (0.8)
MMP-9 (ng/ml)	42.1 (82)	48.2 (51)
TIMP-1 (ng/ml)	192.9 (108)	163.0 (111)

* $p < 0.05$ compared to controls ** $p < 0.0001$ compared to controls. Clinical data shown as mean (SD), MMP and TIMP-1 data shown as median (IQR)

MMP-9 was expressed in the proform and little active enzyme was found (not shown). MMP-2 was not found in any sample (not shown). In the diabetes group, there was no significant difference in MMP-9 or TIMP-1 expression between those who were treated with diet and those receiving oral agents ($p > 0.5$ for both MMP-9 and TIMP-1), or between those taking gliclazide or other sulphonylureas ($p > 0.5$ for both MMP-9 and TIMP-1).

Comparison between groups (Table 1)

Basal median monocyte MMP-1, MMP-3, MMP-9 and TIMP-1 expression did not differ significantly between controls and Type 2 diabetes groups. Age, fasting plasma glucose and HbA1c were not significantly related to MMP or TIMP-1 production in either group (all $p > 0.1$)

Discussion

The main finding of this study is that peripheral venous monocytes from patients with Type 2 diabetes and without clinical coronary artery disease do not differ in MMP-9, MMP-3, MMP-1 or TIMP-1 expression compared to controls. Type 2 diabetes is associated with abnormalities in a number of variables that influence MMP expression in vitro [7,10], but in vivo monocyte MMP and TIMP-1 expression do not appear to be abnormal in Type 2 diabetes. There is surprisingly little data on MMP or TIMP expression in human Type 2 diabetes, despite the great interest in the MMP system and coronary plaque stability. Increased MMP-9 expression in vascular tissue and plasma of animal models of diabetes [16] has been described, and vascular endothelial cells express more MMP-9 in conditions of high glucose in vitro [16], and wound fluid and ulcer punch biopsies derived from diabetes subjects show increased expression of MMP-2, MMP-1 and MMP-8 and conflicting results for MMP-9 [17,18]. However, other groups have described down regulation of an MMP induction and activation system in internal mammary arteries derived from subjects with diabetes [19]. Elevated plasma levels of MMP-9 are a feature of Type 2 diabetes with microabuminuria [20], and in Type 1 patients [21] although it is unclear what relevance plasma MMP concentrations have to any tissue biological process. None of the diabetes subjects in this study were taking aspirin or HMG Co A reductase inhibitors which could have influenced MMP expression [22,23], and although gliclazide may have some antioxidant properties [24], the Type 2 diabetes patients taking gliclazide did not differ significantly in MMP or TIMP expression from those taking no or other medication. It is necessary to stress that these observations were made in Type 2 diabetes patients in good glycaemic control and without clinically expressed coronary artery disease, and it is unknown if monocyte MMP or TIMP expression is different in Type 2 patients with poor control or established vascular disease.

The results from this study cannot be extended to macrophage MMP expression within the coronary plaque or vessel wall after monocyte – macrophage transformation, but it does suggest basal monocyte MMP and TIMP-1 expres-

sion prior to monocyte infiltration and transformation are not abnormal in Type 2 diabetes.

Competing interests
None declared.

Authors contributions
MDB undertook MMP analysis and cell culture work and helped design the study and write the paper ; JG participated in the cell culture and MMP analysis and helped write the paper ; IRD undertook the monocyte separation and helped design the study; DAH helped with monocyte separation and writing the paper; MJS conceived of the study and wrote the final drafts. All authors have seen and approved the final manuscript.

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