Matrix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 and -2 in Type 2 Diabetes

Effect of 1 year's cardiovascular risk reduction therapy

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Cardiovascular risk reduction therapy in diabetic patients reduces vascular events (1) by retarding atherosclerosis and improving vascular reactivity. Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are integral to the vascular changes of atheroma, and MMP-9 and TIMP-1 are raised in diabetes (2). We hypothesized that global risk reduction of intensified diabetes and cardiovascular risk management in type 2 diabetes would be associated with reductions in circulating markers of extracellular matrix turnover, namely MMP-9, TIMP-1, and TIMP-2.

RESEARCH DESIGN AND METHODS

Cross-sectional study

Eighty-six patients with type 2 diabetes (3) and treated hypertension with serum creatinine <120 mmol/l were recruited from local clinics (Table 1). Of these, 46% ($n = 36$) had a clinical history of cardiovascular disease. Data from these patients were compared with 49 healthy control subjects. Ethical approval and informed consent were obtained.

Interventional study

Sixty-five of the recruited diabetic patients (of whom 36 had cardiovascular disease) consented to intensified diabetes and cardiovascular risk management therapy. This consisted of 3 monthly consultations, lifestyle advice, and adjusting oral hypoglycemia therapy to achieve HbA_{1c} <6.5%. Metformin (starting at 500 mg b.d.) was instigated in patients with a BMI >25 kg/m^2 or added second-line in lean patients, who received initial treatment with gliclazide MR (30 mg o.d.) and vice versa. Insulin could be started if HbA_{1c} remained >7.0% despite maximal oral hypoglycemic therapy. Blood pressure reduction was targeted at <130/80 mmHg. All patients were commenced on the ACE inhibitor perindopril (2 mg o.d.), with dose increments or other antihypertensive agents added to achieve goal blood pressure. Statin therapy and 75 mg daily aspirin was advised in all patients. HbA_{1c}, blood pressure, lipid profile, and research indexes were measured at baseline and following 1 year of intensified therapy.

Laboratory analyses

Citrated venous blood was obtained and immediately centrifuged at 1,000*g* and 4°C for 20 min. Plasma was aliquoted and stored at −70°C for a batch analysis of MMP and TIMP by enzyme-linked immunosorbent assay (5).

Power calculations and statistical analysis

We hypothesized a minimum difference of half an SD between case and control subjects. To achieve this at $2p<0.025$ and $1-\beta = 0.85$ required a minimum of 44 case and control subjects. We estimated that the treatment would produce a quarter SD difference in research indexes, and at $2p < 0.025$ and $1-\beta = 0.85$, calculated a minimum requirement of 60 subjects. We used the Mann-Whitney test to compare the control subjects with the pretreatment group and the Wilcoxon's rank-sum test for pre- and posttreatment MMP/TIMP comparisons. Correlations were performed using the Spearman test. A P value of <0.05 was taken as statistically significant.

RESULTS

— Patients and control subjects were of similar age, sex, smoking status, and BMI (Table 1), but baseline systolic blood pressure and HbA_{1c} were higher while HDL was lower in the diabetic patients.

The circulating plasma levels of MMP-9, TIMP-1, and TIMP-2 were all significantly higher in the diabetic patients (Table 1). Within diabetic patients, current smokers ($n = 14$) had higher circulating TIMP-1 levels than nonsmokers (510 ng/ml [335–660] vs. 390 [290–475], $P = 0.017$). There was a weak positive correlation between TIMP-1 and HbA_{1c} ($r = 0.214$, $P = 0.048$) only.

Effects of intervention

Mean serum cholesterol (5.0 ± 1.0 vs. 4.3 ± 0.8 mmol/l, $P < 0.0001$) and HbA_{1c} (7.7 [7.0–8.4%] vs. 7.1 [6.5–7.5], $P = 0.0001$) levels fell significantly; however, blood pressure or BMI did not. Baseline and 1-year usage of aspirin, metformin statins, ACE inhibitors, and additional antihypertensive treatment (i.e., β-blocker, diuretics, calcium channel blockers, α-blocker) were 42 vs. 62%, 42 vs. 68%, 24 vs. 74%, 100 vs. 100%, and 64 vs. 74%, respectively. Only TIMP-1 levels fell significantly (415 ng/ml [375–515] vs. 399 [306–485], $P <$ 0.0001) with intervention.

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Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase.

Table 1—*Baseline characteristics of study population*

	Patients	Control subjects	*P*
n	86	63	—
Age (years)	68 ± 6	66 ± 10	0.115*
Male	67	53	0.079†
Cardiovascular disease	46	—	—
Current smokers	16	12	0.575†
Drug therapy			
ACE inhibitor	100	—	—
Aspirin	42	—	—
Statin	24	—	—
Sulphonylurea	100	—	—
Metformin	43	—	—
Glitazone	3	—	—
BMI (kg/m^2)	28 ± 4	27 ± 4	0.575*
Systolic blood pressure (mmHg)	139 ± 19	131 ± 13	0.006*
Diastolic blood pressure (mmHg)	77 ± 10	76 ± 10	0.578*
Cholesterol (mmol/l)	5.0 ± 1.1	5.3 ± 1.1	0.065*
HDL (mmol/l)	1.3 ± 0.3	1.6 ± 0.4	<0.0001*
Triglycerides (mmol/l)	1.6 (1.2–2.6)	1.5 (0.8–2.2)	0.146‡
HbA_{1c} (%)	7.4 (6.6–8.2)	5.4 (5.2–5.6)	<0.0001‡
TIMP-2 (ng/ml)	143 (120–160)	110 (93–143)	<0.0001‡
MMP-9 (ng/ml)	65 (48–81)	56 (45–75)	0.028‡
TIMP-1 (ng/ml)	397 (300–496)	280 (225–305)	<0.0001‡

Data are means ± SD, median (interquartile range), or percent. *Student's *t* test; †χ^2 analysis; ‡Mann-Whitney *U* test. To convert cholesterol and HDL from mmol to mg/dl multiply by 38.6; to convert triglycerides multiply by 88.5

CONCLUSIONS — Circulating MMP-9, TIMP-1, and TIMP-2 are raised in treated hypertensive patients with type 2 diabetes compared with normotensive control subjects, which is in keeping with extracellular matrix abnormalities present in this high-risk population.

TIMP-1 is an endogenous MMP inhibitor that may be involved in vascular matrix fibrosis (4) and has a role in left ventricular hypertrophy and diastolic dysfunction by reducing cardiac collagen type I turnover, thereby increasing cardiac mass and stiffness (4,5). Increased central and peripheral artery stiffness (6) occurs with diabetes (7), and higher circulating levels of TIMP-1 complement the hypothesis that altered TIMP-1 activities could be linked to arterial stiffness. Smokers have abnormal arterial compliance (8), and raised TIMP-1 levels could mirror increased vascular stiffness. Glucose and cholesterol reduction have both been shown to improve vascular compliance (9,10). The fall in TIMP-1 seen here by improvement in metabolic control raises the possibility of TIMP-1 as a marker of vascular composition in diabetes.

Recent reports have suggested a decrease in renal (11) and vascular tissue MMP-9 (12,13) and an increase in endothelial cell and macrophage MMP-9 in response to glucose (14). Our findings suggest a total increase in MMP-9 in diabetes and that endothelium and macrophages may be partially responsible for this rise. The failure of MMP-9 (and TIMP-2) to fall may relate to the lack of change in blood pressure during intervention. Treatment with insulin (in obese nondiabetic subjects) can reduce MMP-9 and may also explain why circulating MMP-9 did not fall in our study (15).

Diabetes is associated with abnormal angiogenesis (both impaired and/or excessive) and can impair the process of collateralization (16). TIMP-2 may attenuate angiogenesis (17). Increases in circulating TIMP-2 may reflect abnormal blood vessel formation. Manipulating this process may have some therapeutic potential.

A major limitation of this and many other studies is a lack of tissue-based measures. Unfortunately, obtaining human tissue is difficult, and venesection is often the only practical analysis. Furthermore, our patient group is heterogenous, and therefore, it is difficult to show in our group which particular intervention had the most effect. However, since most patients in routine clinical practice show similar profiles to our cohort, this point is of academic rather than clinical interest, as it is rare for such patients to present, and multifactorial intervention is the rule nowadays. This study suggests that further work in either human (in particular vascular compliance, which we were unable to measure) or animal models to further clarify the role of matrix in diabetes is clearly warranted.

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References

1. Gaede P, Vendel P, Larsen N, Jensen G, Parving H, Penderson O: Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 348:383–393, 2003
2. Maxwell P, Timms P, Chandran S, Gordon D: Peripheral blood alterations of TIMP-1, MMP-2, and MMP-9 in patients with type 1 diabetes. *Diabet Med* 18:777–780, 2001
3. World Health Organization: *Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation Part 1: Diagnosis and Classification of Diabetes Mellitus*. Geneva, World Health Org., 1999
4. Tayebjee M, MacFadyen R, Lip GY: Extracellular matrix biology: a new frontier in linking the pathology and therapy of hypertension? *J Hypertens* 21:2211–2218, 2003
5. Lim H, Tayebjee M, MacFadyen R, Lip GY: Tissue inhibitor of matrix metalloproteinase-1 is a marker of left ventricular diastolic dysfunction in diabetic heart disease (Abstract). *J Am Coll Cardiol* 43:221A, 2004
6. Van Bortel L, Struijker-Boudier H, Safar M: Pulse pressure, arterial stiffness, and drug treatment of hypertension. *Hypertension* 38:914–921, 2001
7. Schram M, Henry R, Van Dijk R, Kostense P, Dekker J, Nijpels G, Heine RJ, Bouter LM, Westerhof N, Stehouwer CD: Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension* 43:176–181, 2004
8. Levenson J, Simon A, Cambien F, Beretti C: Cigarette smoking and hypertension: factors independently associated with blood hyperviscosity and arterial rigidity. *Arteriosclerosis* 7:572–577, 1987

9. Kontopoulos A, Athyros V, Pehlivanidis A, Demitriadis D, Papageorgiou A, Boudoulas H: Long-term treatment effect of atorvastatin on aortic stiffness in hypercholesterolaemic patients. *Curr Med Res Opin* 19:22–27, 2003
10. Jensen-Urstad K, Reichard P, Rosfors J, Lindblad L, Jensen-Urstad M: Early atherosclerosis is retarded by improved long-term blood glucose control in patients with IDDM. *Diabetes* 45:1253–1258, 1996
11. McLennan S, Kelly D, Cox A, Cao Z, Lyons J, et al: Decreased matrix degradation in diabetic nephropathy: effects of ACE inhibition on the expression and activities of matrix metalloproteinases. *Diabetologia* 45:268–275, 2002
12. Mondy J, Anstadt M, Franga D, Oortik-Dobos V, Hutchinson J, Ergul A: Decreased vascular matrix metalloproteinase abundance in diabetic patients with symptomatic macroangiopathy. *Ethn Dis* 12 (Suppl. 3):18–22, 2002
13. Portik-Dobos V, Anstadt M, Hutchinson J, Bannan M, Ergul A: Evidence for a matrix metalloproteinase induction/activation system in arterial vasculature and decreased synthesis and activity in diabetes. *Diabetes* 51:3063–3068, 2002
14. Death A, Fisher E, McGrath K, Yue D: High glucose alters matrix metalloproteinase expression in two key vascular cells: a potential impact on atherosclerosis in diabetics. *Atherosclerosis* 168:263–269, 2003
15. Dandona P, Aljada A, Mohanty P, Ghanim H, Bandyopadhyay A, Chaudhuri A: Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care* 26:3310–3314, 2003
16. Martin A, Komada M, Sane D: Abnormal angiogenesis in diabetes mellitus. *Med Res Rev* 23:117–145, 2003
17. Haas T, Madri J: Extracellular matrix-driven matrix metalloproteinase production in endothelial cells: implications for angiogenesis. *Trends Cardiovasc Med* 9: 70–77, 1999