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Diabetes Research and Clinical Practice 71 (2006) 45–51
DIABETES RESEARCH AND CLINICAL PRACTICE
www.elsevier.com/locate/diabres

Monocyte matrix and ADAM metalloproteinase expression in type 2 diabetes after aspirin therapy

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Received 5 October 2004; received in revised form 8 March 2005; accepted 20 May 2005
Available online 14 July 2005

Abstract

The matrix metalloproteinase system (MMP and the TIMP inhibitors), and the ADAM metalloproteinases, have roles in maintaining vascular plaque stability and the shedding of cell surface molecules, such as TNF-α and adhesion molecules; aspirin suppresses MMP expression and ADAM activity from some cell lines in vitro. In a randomised prospective controlled study, we examined peripheral venous monocyte MMP-9, TIMP-1 and ADAM mRNA levels, and protein expression, in subjects with type 2 diabetes ($n = 10$) and controls ($n = 14$) before and after oral aspirin therapy (150 mg daily for 14 days) or no active intervention.

Baseline monocyte TIMP-1 mRNA levels were significantly lower in the diabetes group ($p = 0.0014$), although monocyte MMP-9 mRNA, and MMP-9 and TIMP-1 protein expression after culture did not differ significantly between groups. Plasma MMP-9 ($p = 0.027$) and TIMP-1 ($p = 0.016$) concentrations were significantly greater, and the ratio of plasma TIMP-1:MMP-9 concentrations significantly lower, in the diabetes group ($p = 0.023$). ADAM mRNA levels did not differ significantly between groups and oral aspirin therapy had no significant effect on any variable.

Type 2 diabetes is characterised by reduced monocyte TIMP-1 mRNA levels, and a lower plasma MMP-9 to TIMP-1 protein ratio compared to controls, a pattern that would promote coronary plaque instability if reproduced within vascular plaque. Monocyte ADAM mRNA levels do not differ between group and oral aspirin has no significant effect on these variables.

Keywords: Type 2 diabetes; Aspirin; Matrix metalloproteinases; ADAM metalloproteinases

1. Introduction

The matrix metalloproteinases (MMP) are a family of proteinases capable of degrading all extracellular matrix components and whose activity is tightly

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doi:10.1016/j.diabres.2005.05.014

controlled by endogenous tissue inhibitors (TIMP) [1,2]. Localised overexpression or unbalanced expression of MMP within vascular plaque may increase the risk of plaque instability, plaque rupture, and an acute vascular event [1–3]. Many of the variables shown in vitro to contribute to increased MMP expression from a range of cell lines are abnormal in type 2 diabetes [4–7], and abnormal in a way that should theoretically promote increased MMP expression in vivo in type 2 diabetes. The evidence for this in vivo in human diabetes is limited, and although MMP-2 and MMP-3 expression from normal dermal fibroblasts in human diabetes is increased [8], monocyte MMP and TIMP protein expression ex vivo in type 2 diabetes does not appear to be abnormal [9]. A further family of metalloproteinases possess a disintegrin and metalloproteinase domain (ADAM) and are involved in the shedding of active forms of cell surface bound proteins, such as TNF-α [10], soluble adhesion molecules, such as l-selectin, VCAM-1 and XVII collagen [10–13], and are also involved in cell–cell adhesion [14]. Elevated plasma levels of TNF-α and the soluble forms of many adhesion molecules are characteristic of type 2 diabetes [15–18] and must, to some degree, reflect the shedding and release of these soluble forms from the transmembrane bound form by ADAM metalloproteinases. The ADAM metalloproteinases have not been studied in human diabetes.

Aspirin is a widely used therapy for cardiovascular event prevention, a vascular benefit mediated through inhibition of platelet aggregation and adhesion, anti-inflammatory and anti-oxidant actions, and enhanced fibrinolysis [19]. Aspirin also reduces the expression of certain MMPs, at least from some tumour cell lines in vitro [20–22] which could potentially be a mechanism for vascular benefit if reproduced in vivo in vascular cells. Non-steroidal anti-inflammatory agent also inhibit the function of the TNF-α converting enzyme (TACE or ADAM17) in vitro [23]. As far as we aware there are no data on MMP, TIMP or ADAM mRNA expression from primary cell lines in human type 2 diabetes, or on the impact of oral aspirin on these variables. We studied monocyte MMP and ADAM mRNA expression in type 2 diabetes and controls before and after oral aspirin therapy in a randomised controlled trial.

2. Methods

2.1. Subjects

Subjects with type 2 diabetes ($n = 10$) were recruited if they were male non-smokers with type 2 diabetes and no clinical history or electrocardiographic evidence of ischaemic heart disease. Controls without type 2 diabetes ($n = 14$) were recruited if they were male non-smokers with no electrocardiographic evidence of ischaemic heart disease and a fasting plasma glucose below 6.1 mmol/L. No patient or control was taking aspirin or lipid lowering therapy. Six of the type 2 patients were managed with metformin alone, two with diet alone, and two with a combination of a sulphonylurea and metformin. In addition, three type 2 diabetes patients were taking an ACE inhibitor and a thiazide diuretic for blood pressure control. No subject with type 2 diabetes had microalbumiuria or macroproteinuria. None of the controls were taking any medication. All subjects and controls were Caucasian. Patients were randomised to take 150 mg of aspirin daily or a matched placebo for 14 days, and six of the 14 controls, and six of the 10 type 2 diabetes patients received aspirin.

2.2. 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 [24]. Monocyte purity was greater than 85% and cell viability (assessed by trypan blue exclusion) was normally greater than 95% [24]. Paired analyses were undertaken on samples taken at time 0, and after 14 days of aspirin therapy.

2.3. Monocyte culture

The monocytes were re-suspended in RPMI 1640 medium (Gibco) containing 10 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 h, 37 °C, 5% CO_2, at a density of 250,000/well in 24-well tissue culture dishes (Costar), in a total volume of 0.5 ml. After 48 h,