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Original article

Microparticle Shedding by Erythrocytes, Monocytes and Vascular Smooth Muscular Cells Is Reduced by Aspirin in Diabetic Patients

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ABSTRACT

Introduction and objectives: Diabetes mellitus is associated with an enhanced risk for cardiovascular disease and its prevalence is increasing. Diabetes induces metabolic stress on blood and vascular cells, promoting platelet activation and vascular dysfunction. The level of vascular cell activation can be measured by the number and phenotype of microparticles found in the circulation. The aim of this study was to investigate the effect of a platelet-inhibitory dose of aspirin on the number and type of microparticles shed to the circulation.

Methods: Forty-three diabetic patients were enrolled in the study and received a daily dose of 100 mg of aspirin for 10 days to cover the average platelet life-span in the circulation. Before and after the intervention period, circulating microparticles were characterized and quantified by flow cytometry.

Results: Type 1 diabetic patients had about twice the number of tissue factor-positive circulating microparticles (derived both from platelets and monocytes) and endothelial-derived E-selectin positive microparticles than type 2 diabetic patients. Aspirin therapy significantly inhibited platelets since cyclooxygenase 1 derived thromboxane generation levels were reduced by 99%. Microparticles derived from erythrocytes, activated monocytes, and smooth muscle cells were significantly reduced after 10 days of aspirin administration.

Conclusions: These results indicate that: *a)* vascular and blood cells in type 1 diabetic patients are exposed to more sustained stress shown by their specific microparticle origin and levels; *b)* aspirin therapy inhibits vascular wall cell activation and microparticle shedding, and *c)* the effects of aspirin are similar in type 1 and 2 diabetes.

El ácido acetilsalicílico reduce la liberación de micropartículas eritrocitarias, monocitarias y de células del músculo liso vascular en pacientes diabéticos

Palabras clave:
Diabetes mellitus
Ácido acetilsalicílico
Micropartículas circulantes derivadas de células
Factor tisular
Células activadas

RESUMEN

Introducción y objetivos: La diabetes mellitus está asociada a mayor riesgo de enfermedad cardiovascular y su prevalencia está en aumento. La diabetes mellitus induce estrés metabólico a las células vasculares, lo cual promueve la activación plaquetaria y la disfunción vascular. El grado de activación de las células vasculares se puede medir con el número y el fenotipo de las micropartículas circulantes. El objetivo de este estudio es investigar el efecto de una dosis de ácido acetilsalicílico con acción inhibidora plaquetaria en el número y el tipo de las micropartículas liberadas a la circulación.

Métodos: Se inscribió a 43 pacientes diabéticos que recibieron una dosis diaria de 100 mg de ácido acetilsalicílico durante 10 días, con objeto de cubrir el periodo medio de vida de las plaquetas en la circulación. Antes y después del periodo de intervención, se caracterizaron y cuantificaron las micropartículas circulantes mediante citometría de flujo.

Resultados: Respecto a los pacientes con diabetes mellitus tipo 2, aquellos con diabetes mellitus tipo 1 presentaron el doble de micropartículas circulantes positivas para factor tisular (derivadas de plaquetas y monocitos) y micropartículas positivas para selectina E de origen endotelial. El tratamiento con ácido acetilsalicílico inhibió significativamente las plaquetas, puesto que la generación de tromboxano derivado de la ciclooxigenasa 1 disminuyó un 99%. Se produjo una reducción significativa de las micropartículas derivadas de eritrocitos, monocitos activados y células de músculo liso tras 10 días de administración de ácido acetilsalicílico.

Conclusiones: Estos resultados indican que: *a)* las células vasculares y hemáticas de los pacientes con diabetes mellitus tipo 1 están expuestas a un mayor estrés continuo, que se refleja en el origen y la

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cantidad de sus micropartículas; *b)* el tratamiento con ácido acetilsalicílico inhibe la activación de las células de la pared vascular y la liberación de micropartículas, y *c)* los efectos del ácido acetilsalicílico son similares en la diabetes mellitus tipos 1 y 2.

Abbreviations

AV: annexin V
cMPs: circulating microparticles
CVD: cardiovascular disease
DM: diabetes mellitus
TF: tissue factor

INTRODUCTION

Diabetes mellitus (DM) is largely associated with microvascular and macrovascular complications and an enhanced risk of cardiovascular disease (CVD).[1] Because of the increasing prevalence of DM worldwide, it has been estimated that approximately 552 million people will have DM in 2030, of whom more than 95% will have type 2 DM (DM2).[2] A number of mechanisms for the increased cardiovascular (CV) risk in DM have been proposed, including an increased tendency toward intracoronary thrombus formation, increased platelet reactivity, and worsened endothelial dysfunction.[3] Because up to 80% of individuals with DM will die of CV causes, evidence-based therapies to reduce CVD are of the utmost importance.

The recommendation of low-dose aspirin for the primary prevention of CV events in adults with DM has been the subject of controversy.[2,3] Aspirin is a nonsteroidal anti-inflammatory drug and is the most commonly used antiplatelet agent due to its low cost and relative lack of adverse effects when administered in low doses. Aspirin inhibits platelet thromboxane A_2 formation, a potent vasoconstrictor and platelet agonist, through the acetylation of cyclooxygenase 1 in the serine-530 position, thus preventing the arachidonic acid binding to the enzymatic active site.[4,5] Beyond the inhibitory effect on thromboxane formation, aspirin may have pleiotropic effects, involving antioxidant and anti-inflammatory effects,[6] but the effects of aspirin on preventing cell activation from the vascular compartment and microparticle shedding still remain unclear.

Circulating microparticles (cMPs) are small phospholipid microvesicles of 0.1 to 1 μm diameter, shed by activated endothelial or blood cells and defined by both size and expression of cell-specific antigens on their surface.[7] Recent studies have shown that these cMPs play a key role in thrombosis, inflammation and angiogenesis,[8,9] which are essential in the development of diabetic complications. Although present in the plasma of healthy individuals, elevated numbers of specific activated subsets of cMPs have been reported in vascular disorders.[10–13] Circulating microparticles originate from cells and contain phosphatidylserine and distinct surface proteins depending on their cells of origin or parental cells. Circulating microparticles can originate from platelets, endothelial cells, leukocytes, erythrocytes, and smooth muscle cells.[14] Some have strong procoagulant properties due to exposure of anionic phospholipids, such as phosphatidylserine, in a similar fashion as activated platelets, and provide a catalytic surface that may promote coagulation since phosphatidylserine facilitates the binding of the coagulation factors and the assembly of the coagulation complexes, accelerating the formation of thrombin.[15] As reviewed,[16] patients with DM and diabetic complications have different cellular cMPs patterns, and blood levels of platelet-derived cMPs, endothelial-derived cMPs, and total annexin V $(AV)^+$ microparticles are significantly increased in type 1 DM (DM1). In DM2 patients, higher levels of total,[17] platelet, leukocyte, monocyte, and endothelial-derived cMPs have been observed compared with those of matched controls.[18–21]

Thus, the aim of this study was to determine the effect of aspirin administration on microparticle shedding and phenotype in diabetic patients.

METHODS

Diabetic Patients

A total of 43 primary care diabetic patients (men and women) aged between 41 and 73 years and treated according to international guidelines were recruited for the study in the outpatient clinic of the *Hospital de la Santa Creu i Sant Pau* in Barcelona, Spain. The patients included in the study had DM1 or DM2, and exclusion criteria were as follows: aspirin or ibuprofen ingestion within the last 10 days, contraindications to aspirin or beta-blocking agents, peptic ulcer and increased bleeding risk as well as a past history of cancer, inflammatory disorders, sepsis, infection, or pregnancy.

The study protocol was approved by the institutional review board of the hospital, and the trial was conducted according to the Declaration of Helsinki. All participants gave written consent before participation in the study. After screening and inclusion in the study, a medical record was administered to obtain lifestyle, medical and therapeutic data, and baseline measurements were performed. After that, patients were entered at the intervention period, when they were administered 100 mg aspirin daily for 10 days. Blood samples were taken before (baseline) and after the intervention period with aspirin.

To ensure adherence to aspirin therapy and its efficacy, cyclooxygenase-1 inhibition was proven by measuring the inhibition of thromboxane B_2 formation with a commercial enzyme immunoassay kit (Thromboxane B2 Express Eia Kit-Monoclonal, Cayman Chemical) following manufacturer's recommendations.

Control Participants

As control participants, we included 38 moderate-high CV risk participants free of DM and CVD matched for sex, age, classical CV risk factors, and statin use. Controls were recruited from the SAFEHEART cohort,[22] an open, multicenter, long-term prospective study. None of the control participants were undergoing aspirin therapy. Data on demographic and clinical characteristics, CV history, classic CV risk factors, and current treatment for hypercholesterolemia were obtained from all participants using a standardized report form at inclusion. The study was approved by the local ethics committee and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the study.

Blood Sampling

Venous blood was drawn from the cubital vein without tourniquet using a 20-gauge needle after 10-14 hours of fasting