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Gender-related drug effect on several markers of oxidation stress in diabetes patients with and without complications

Maura Lodovici [a,*], Elisabetta Bigagli [a], Cristina Luceri [a], Edoardo Mannucci [b], Carlo Maria Rotella [c], Laura Raimondi [a]

[a] Department of NEUROFARBA, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy
[b] Diabetes Agency, Careggi Teaching Hospital, Florence, Italy
[c] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

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ABSTRACT

We previously reported that circulating lipid (malondialdehyde, MDA) and protein oxidation (carbonyl residues, CO) products can be used as markers of risk for complications in poorly controlled type 2 diabetics. Now, we aimed to evaluate the existence of a gender effect on classical disease markers and oxidative stress parameters and on the effectiveness of metformin and/or statins in reducing CV risk in poorly controlled type 2 diabetics with and without complications. Our results show that diabetics with complications had higher plasma levels of FRAP, SOD and hs-CRP than those without complications, with FRAP and SOD found increased in both genders. Interestingly, male and female patients with complications had higher plasma levels of hs-CRP and MDA respectively, over patients without complications. Multivariate analysis indicated metformin and statin treatments effective in reducing plasma hs-CRP only in female and not in male diabetics with complications. In these latter females, a positive correlation between hs-CRP and triglycerides (TG) levels was found suggesting a causal relationship between them. Statin treatment was effective in reducing MDA in diabetics with complications irrespective of the gender. These data support the addition of statins to diabetic standard therapy to control oxidation injury and inflammation and, for the first time, indicate female patients with complications more responsive than males to the CV protection offered by metformin.

1. Introduction

Type 2 diabetes is characterized by insulin resistance and/or impaired insulin secretion, producing chronic hyperglycemia which causes the accumulation of reactive oxygen species produced by different pathways (Robertson and Harmon, 2006). While low reactive oxygen species levels participate in maintaining cardiac and vascular integrity, elevated reactive oxygen species levels are recognized as among the pathogenic events of the cardiovascular dysfunction associated with diabetes (Rask-Madsen and King, 2007).

An imbalance between increased production and reduced scavenging of reactive oxygen species leads to a metabolic state of oxidative stress, triggering tissue damage. On this basis, Vitamin E and other antioxidants were proposed as treatments to prevent diabetes complications, but, unfortunately, these compounds did not reveal any significant long term beneficial effects (Levy et al., 2004; Blum et al., 2010).

The pharmacological treatment of diabetic patients is crucial for delaying the progression of the syndrome and of its complications; however, differences in the effectiveness of drug-related cardiovascular (CV) protection were reported (Sarwar et al., 2010). Among the first line treatment of type 2 diabetes and its cardiovascular complications are metformin (Stern et al., 2015) and statins (De Vries et al., 2014). The beneficial effects of these drugs include the ability to oppose diabetes-induced damaging oxidants (Vitale et al., 2005; De Aguiar et al., 2006; Manfredini et al., 2010) a finding which allows us to consider these treatments as "antioxidants". Until now, whether the protection offered by these drugs is similar in men and women is unknown. This question is of particular interest, since CV risk associated with diabetes is higher in men than in premenopausal women (ESHRE Capri Workshop Group, 2006) while gender-based differences are lost following menopause (Rossouw et al., 2002).

We previously reported that the increase in circulating lipid and/or protein oxidation products can be used as a marker for detecting patients at high risk of developing complications in poorly controlled type 2 diabetes (Bigagli et al., 2011).

* Corresponding author.
E-mail address: maura.lodovici@unifi.it (M. Lodovici).

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In poorly controlled type 2 diabetic patients with and without complications, we now aimed to evaluate the existence of a gender effect on (i) classical disease markers and oxidative stress parameters and (ii) the effectiveness of metformin and/or statins treatments in CV protection.

2. Materials and methods

2.1. Patients

Eighty-nine consented type 2 diabetic patients with poor glycemic control (glycated haemoglobin A1c (HbA1c) > 7.0%, > 53 mmol/mol; fasting glucose > 7.78 mmol/l) were included in an observational study, from the diabetology unit of the Department of Experimental and Clinical Medicine, University of Florence and the Diabetes Agency, Careggi Teaching Hospital, Florence, Italy. Among the exclusion criteria were HbA1c levels = or < 7% and the lack of informed consent. The study was approved by the local hospital ethics committee. Written informed consent was obtained from all patients. They were divided into two groups: one group consisted of 52 patients (32 males and 20 females) with type 2 diabetes without complications and the second group consisted of 37 patients (15 males and 22 females) with complications (micro and macrovascular). The patients' history of coronary heart disease, peripheral vascular disease, diabetic neuropathy and diabetic retinopathy was collected from the hospital records. All patients had undergone at least one in-depth examination, an electrocardiogram, measurement of Ankle Brachial Index (ABI), urinary albumin excretion rate (AER) and serum creatinine in the 12 months prior to enrollment. Retinopathy was diagnosed on fundal examination for the presence of micro aneurysms, dot and blot hemorrhages or retinal changes such as edema and thickening. Nephropathy was diagnosed on clinical examination and the presence of proteinuria with at least two values of albumin creatinine ratio (ACR) > 30 mg/g. If proteinuria was found to be negative, then microalbuminuria was assessed by dipstick (Micral dipstick, Roche Diagnostic). The presence of macro-vascular complications was evaluated by cerebral vascular accidents (CVA)/stroke, coronary artery disease (CAD) and peripheral vascular disease (PVD). The presence of CAD was based on electrocardiographic changes suggestive of ST segment depression, Q2 wave changes and/or T wave inversion using the appropriate Minnesota code, or a positive treadmill test/stress echocardiography or positive coronary angiography wherever available. Peripheral vascular disease (PVD) was assessed based on the clinical findings of decreased peripheral arterial pulsation and/or an ankle/brachial index of < 0.9 on vascular doppler wherever available. Medications taken by diabetic patients were as follows (non-exclusive): 2nd generation sulphonylureas (2.5–5 mg/day, $n=20$), biguanides (metformin, used in the range dose of 1.5–3 g/day, $n=59$), insulin ($n=42$), thiazolindiones (15–45 mg/day, $n=6$) and glinides (1–12 mg/day, $n=7$). Of the patients enrolled, 50 were solely treated with oral drugs, 6 treated solely with metformin, 2 with insulin alone and the others ($n=38$) were treated with insulin in addition to oral drugs. Moreover, 38 patients were also treated with statins (including pravastatin (10–40 mg/day), simvastatin (10–20 mg/day), torvastatin (10–80 mg/day) and rosuvastatin (5–40 mg/day)) and 58 were treated with antihypertensive drugs.

Venous blood samples were taken from each patient and, collected into EDTA-treated tubes; and all of the plasma was stored at −20 °C until analysis, which was done within 30 days. HbA1c was analyzed by an automatic analyzer (Backman Coulter, Italy).

2.2. Biochemical analyses

2.2.1. Chemicals and equipment

All chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO, USA) and were of analytical grade or of the highest grade available.

2.2.2. Measurement of Hs-CRP

hs-CRP level was determined by the high sensitivity assay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA) (Lubrano et al., 2005).

2.2.3. Measurement of superoxide dismutase (SOD)

The total SOD activity (Cu/Zn and Mn-SOD) was determined in the plasma by using the nitro blue tetrazolium reaction (NBT) (Beauchamp and Fridovich, 1971). The method is based on the capacity of SOD to inhibit NBT reduction. The color reaction was measured by NanoPhotometer (Implen, Munchen) at 560 nm.

2.2.4. Malondialdehyde (MDA) levels, carbonyl residues (CO) and ferric reducing ability of plasma (FRAP) level measurements

MDA was determined after derivatization with dinitrophenylhydrazine as described by Mateos et al. (2004). CO levels were determined by the method of Correa-Salde and Albesa (2009). The FRAP values were measured according to the method used by Benzie and Strain (1996). All determinations were performed in the plasma.

2.3. Statistical analysis

Data were expressed as means ± S.E.M. Parametric variables were compared using one-way ANOVA. Multiregression analysis was done to evaluate the association between each biomarker of oxidation stress (MDA, FRAP, CO and SOD) including hs-CRP, and independent variables (Total cholesterol (CT), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TG) etc.) or confounding variables including sex, age, diabetes duration and drug treatment such as metformin, sulphonylureas, glinides, insulin, statins and antihypertensive. Thus, independent variables were tested against each biomarker of oxidation stress and hs-CRP. Results were performed using Statgraphics Statistical Packages (Statistical Graphic Corporation, Rockville, MD). The entry value for each independent variable was 0.15.

3. Results

3.1. Anthropometric, clinical and oxidative stress parameters in diabetic patients without and with complications

Table 1 shows the clinical features of the analyzed 52 poorly controlled diabetics (32 males and 20 females without and 15 males and 22 females with complications). All the females included in the study were post-menopausal. Male and female patients had similar drug treatment, the only difference was represented by the percentage of patients received insulin (29% of females and 38% males).

In diabetics without complications, our analysis indicated the absence of gender-related differences in the classic markers of disease, including hs-CRP, in lipid (MDA) and protein (CO) oxidation damage, in enzymatic (SOD) and non-enzymatic (FRAP) circulating anti-oxidative capacity. On the contrary, to note, females without complications had HDL levels significantly ($P<0.05$) higher than male patients.

Table 1 also shows the anthropometric, clinical and oxidative stress parameters of diabetics with complications. It is worth