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Altered glutathione system is associated with the presence of distal symmetric peripheral polyneuropathy in type 2 diabetic subjects

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ABSTRACT

Distal symmetric peripheral polyneuropathy (DSPN) is a highly prevalent complication of diabetes. However, underlying pathophysiological mechanisms are multiple and not well understood. The aim of our study was to analyze the oxidative stress levels in circulating mononuclear cells by measuring the glutathione system, malondialdehyde and oxidized-LDL, in 60 type 2 diabetic patients from a well-characterized cohort of 196 type 2 diabetic patients. Using a nested case–control design, we studied 30 type 2 diabetic patients with distal symmetric polyneuropathy and 30 diabetic controls without this complication, according to the Neuropathy Disability Score. We have found that diabetic patients with distal symmetric polyneuropathy showed significantly lower values of reduced glutathione (GSH) and reduced glutathione/oxidized glutathione (GSH/GSSG) ratio. These data indicate an increased consumption of glutathione in mononuclear cells from patients with distal symmetric polyneuropathy. No significant differences were found in malondialdehyde or in oxidized-LDL levels comparing both groups. These data show an altered glutathione response in circulating monocytes from diabetic patients with distal symmetric polyneuropathy.

1. Introduction

Distal symmetric peripheral polyneuropathy (DSPN) is a highly prevalent complication of diabetes mellitus. Signs and symptoms of peripheral nerve dysfunction in a patient with diabetes characterize DSPN, once other causes of peripheral polyneuropathy have been excluded. Signs and symptoms should begin distally in the feet, on both sides and be primarily sensory (including numbness, prickling paresthesias, dysesthesias, burning or allodynia), motor (such as weakness, atrophy or depressed tendon reflexes) or both. (England, Gronseth, Franklin, et al., 2005)

In addition, DSPN is the most important causal mechanism leading to foot ulceration, a significant cause of morbidity and mortality in diabetic patients (Cheer, Shearman, & Jude, 2009). However, pathophysiological mechanisms underlying the development of diabetic DSPN are multiple and not well understood (Pascuzzi, 2009).

The etiology of diabetic DSPN seems to be heterogeneous, but previous data strongly suggest that hyperglycemia-induced cellular damage and an increased production of reactive oxygen species play major roles in the development of this disease (Brownlee, 2005). Different evidences suggest that oxidative stress (OS) is implicated in the pathogenesis of diabetic neuropathy (Bandeira, da Fonseca, Guedes, et al., 2013; Brownlee, 2005).

Experimental studies have associated polyneuropathy with intracellular hyperglycemia and succeeding altered polyols pathway and increased intracellular advanced glycosylation end products formation, which lead to reactive oxygen species-induced DNA damage (Aubert, Michel, Gillery, et al., 2014; Gabbay, Merola, & Field, 1966; Sugimoto, Yasujima, & Yagihashi, 2008). In the presence of hyperglycemia increased OS is detected in peripheral nerves (glial), dorsal nerves and vascular nerve endothelial cells (Nishikawa, Edelstein, Du, et al., 2000; Pop-Busui, Sima, & Stevens, 2006; Schmeichel, Schmelzer, & Low, 2003). Moreover, the presence of decreased reduced glutathione (GSH) levels and increased oxidized lipoproteins is associated to nerve damage, vacuolization of mitochondria and neuronal apoptosis (Vincent, Russell, Low, & Feldman, 2004). This pathological state induces a reduction in nerve conduction, an altered neurotrophy and neuronal apoptosis; all typical findings of diabetic polyneuropathy (Obrosova, 2002).

Conflict of interest: There is no conflict of interest to declare.

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Unfortunately human studies are scarce. In clinical studies, an increase in perioxinitrate and oxidized lipoproteins levels in diabetic patients with polyneuropathy compared to diabetic subjects with no polyneuropathy has been found; supporting the role of OS in DSPN (Kasznicki, Kosmalski, Sliwinska, et al., 2012). We have recently shown that plasma homocysteine levels, an independent risk factor for cardiovascular disease, are increased in diabetic patients with DSPN and relate with the grade of peripheral neuropathy (Gónzalez, Pedro, Martinez-Hervas, et al., 2012). Moreover, a decreased total antioxidant status in type 2 diabetic patients (T2DM) with coexisting DSPN has been reported (Kasznicki et al., 2012; Merzouk, Hichami, Madani, et al., 2003); although not all investigators support this observation (El Boghdady & Badr, 2012).

Therefore, experimental data strongly implicate OS in the pathogenesis of diabetic polyneuropathy, but there is a need for stronger evidence from clinical studies. Besides, an early appropriate treatment of DSPN would be crucial for the patient's prognosis and could prevent or delay this complication. However, no causative treatment is known since the pathogenesis of DSPN is not fully elucidated (Lavery, Hunt, Lafontaine, et al., 2010).

Few clinical studies have evaluated OS levels in T2DM patients with DSPN; and little is known about the glutathione system, malondialdehyde (MDA) or oxidized low-density lipoprotein (oxidized-LDL). Therefore, the aim of this study was to analyze the OS levels in circulating mononuclear cells by measuring MDA, oxidized-LDL and the glutathione system, in a well-characterized cohort of T2DM patients. We assumed that the consumption of relevant intracellular antioxidant factors (such as reduced glutathione [GSH]) or an increase in OS markers (oxidized glutathione [GSSG], MDA and oxidized-LDL) would be associated with the presence of DSPN in T2DM patients.

2. Subjects and methods

2.1. Subjects

We studied 196 non-related T2DM patients, selected by consecutive sampling at the Diabetic Unit of our center and at two primary health care centers (45% of patients); all subjects were from the metropolitan area of Valencia, Spain.

A nested case–control study was conducted. The cases and controls proceeded from a well-characterized cohort of 196 T2DM. The study included 30 T2DM patients with DSPN and 30 diabetic controls without DSPN who were matched to cases on gender, age, body mass index (BMI), HbA1c and renal function. This was done in a stepwise way to obtain similar age, gender distribution, BMI, HbA1c and glomerular filtration rate (GFR). It is well known that all these parameters can influence redox status, and we use this strategy in order to minimize the effect of these confounding factors (Aranda, Doménech, Rus, et al., 2007; Jones, 2006; Lodovici, Giovannelli, Pitozzi, et al., 2008; Miller, Michael De Silva, Jackman, & Sobey, 2007).

Inclusion criteria were as follows: adult patients (aged ≥ 40 years) with type 2 diabetes (identified based on fasting glucose and/or HbA1c or a prescription for oral or subcutaneous hypoglycemic drugs or insulin). Exclusion criteria were type 1 diabetes, heart failure NYHA class III or IV, renal disease (GFR < 30 ml/min/1.72 m^2), cirrhosis, vitamin supplementation, vitamin B12 or folate deficiency, hypothyroidism, positive HIV, systemic illness, cancer, smoking habit, consumption of >30 g alcohol/day or any other disease, condition or drugs known to cause neuropathy. Patients with Charcot´s arthropathy and/or bilateral amputation were also excluded.

All patients continued on their usual hypoglycemic treatment, as well as drug treatment for other cardiovascular risk factors. The ethical committee of our hospital approved the study and all patients gave their written informed consent.

We also have studied a control group of 55 healthy volunteers ranging age from 18 to 65 years that were randomly recruited among plasma donors and investigators of our center. The inclusion criteria for the control group were: BMI < 30 kg/m^2, concentration of plasma total cholesterol (TC) < 200 mg/dl and triglycerides (TG) < 150 mg/dl; fasting plasma glucose < 110 mg/dl and absence of personal or family history of dyslipidemia, cardiovascular disease or diabetes.

2.2. Clinical and anthropometric parameters

Complete medical history and physical examination were carried out in all the patients. Blood pressure was measured in the sitting position with a mercury sphygmomanometer after a 10-min rest, with two separated measurements; body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared and abdominal circumference was measured in cm in the point between the low costal rim and the iliac crest. The same investigator performed all measurements.

2.2.1. Assessment of distal symmetric polyneuropathy

The neuropathy disability score (NDS) was used to quantify the presence and severity of DSPN obtained from physical examination (Boulton, 1998). An NDS score of 3 or greater was considered abnormal (presence of DSPN).

We also evaluated the loss of protective sensation through application of the 10 g Semmes–Weinstein monofilament (SWM). Three plantar sites on each foot were explored: great toe and base of first and fifth metatarsals. The monofilament was not applied on areas of calluses or other structural abnormalities. A score of 1 was given on each site explored if the patient perceived the force applied in at least 2 of 3 attempts. A score of 0 was given if there was lack of perception. A summation of all scores on both feet between 0 and 4 was considered abnormal, while scores 5 or 6 were normal (Feng, Schlösser, & Sumpio, 2009). The same experienced physician performed all procedures in each participant.

2.2.2. Assessment of peripheral vascular disease

Peripheral vascular disease was assessed through the ankle brachial index (ABI). ABI is the ratio of systolic blood pressure in the ankle to that in the brachial artery. Measurement of ABI is made in supine position, using a hand-held continuous-wave Doppler ultrasound (Bi-directional Smartdrop™ 20). PVD is defined by an ABI < 0.9 in either leg (American Diabetes Association, 2003). Scores above 1.2 were excluded from the statistical analysis due to possible arterial stiffness and the inability to accurately assess the grade of peripheral vascular disease.

2.2.3. Biochemical parameters

Blood samples were drawn following an overnight 12 h fasting period. HbA1c was measured using a high-performance liquid chromatography assay. Total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C) and apolipoprotein B were determined using standard methods previously described (Ascaso, Merchante, Lorente, et al., 1998). Urinary albumin concentration was determined by a standard radioimmunoassay. Glomerular filtration rate was calculated using the Modification of Diet in Renal Disease equation (MDRD).

Markers of OS were determined in circulating mononuclear cells isolated by Ficoll-Hypaque methods, as previously reported (Oltra, Carbonell, Tormos, Iradi, & Sáez, 2001). Oxidized glutathione and reduced glutathione (GSSG and GSH) were determined using high performance liquid columns (HPLC) and UV detection (Navarro, Obrador, Pellicer, et al., 1997). MDA was analyzed by HPLC (Wong, Knight, Hopfer, et al., 1987). Oxidized-LDL was measured by commercial enzyme-linked immunosorbent assay (Biomedica, Wien, Austria).