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Abstract

Charcot-Marie-Tooth disease (CMT) (ORPHA166) is the most frequent hereditary neuropathy. CMT is a heterogeneous group of disorders which, despite some variability in their clinical features, share the same general phenotype, usually characterized by wasting and weakness of distal limb muscles, decreased or absent deep tendon reflexes, distal sensory loss, and frequent skeletal deformities. Despite the clinical and molecular description of this disease in the last 20 years, there is no effective drug or advanced therapy available. Here we have pretend the identification of metabolic and oxidative stress biomarkers in plasmas from patients with duplication at PMP22 gene, the most frequent mutation causing CMT, and clinically characterized as CMT1A. The samples were collected in the neuropathy units from “La Fe” Hospital of Valencia, “Bellvitge” Hospital of Barcelona, “La Paz” Hospital of Madrid, and “Virgen del Rocío” Hospital of Sevilla. The metabolic biomarkers research was performed using 2D-DIGE analysis (Typhoon TRIO, GE) and DeCyder software (GE). Protein identification was made by mass spectrometry by MALDI-TOF-TOF (ABSciex) and liquid Chromatography analysis (ABSciex). The oxidative stress biomarkers research consisted in carbonylated proteins analysis by reaction with DNPH and Dot-blot. Total antioxidant capacity and GSSG/GSH ratio were analyzed with Antioxidant Assay kit (Cayman) and Glutathione Fluorescent detection Kit (Arbor Assays), respectively. Finally now we are performing the MDA levels by HPLC-UV.

We found 8, 13 and 36 proteins with differential expression in mild, moderate and severely affected patients, respectively compared with their own matched controls. Also we found differences on oxidative stress parameters between de different groups analyzed. Our results suggest differences in the oxidative stress profile between the studied phenotypes in CMT1A patients.

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15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ as a potential TRPV1-dependent atopic dermatitis enhancer

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Abstract

Atopic dermatitis (AD) is a recurrent chronic inflammatory skin condition characterized by a complex pathogenesis, including skin barrier dysfunctions, allergy/immunology, and pruritus. In AD lesions, mast cells migrate into the epidermis, and exert their biological effects by releasing paracrine mediators. TRPV1, a non-selective cation channel widely expressed in skin tissues, has been shown to contribute to the development of diverse dermatoses and pruritus. In the present study, we identified a TRPV1 agonist as a neuritogenic enhancer produced from mast cells and characterized a possible molecular mechanism for the

TRPV1-dependent neuritogenesis in AD. Based on the hypothesis that activated mast cells produce a TRPV1-dependent neuritogenic enhancer, we screened a number of inflammatory mediators for the neuritogenic-promoting activity and identified a PGD₂ metabolite, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), as a potential neuritogenic enhancer derived from mast cells. 15d-PGJ₂ significantly enhanced the nerve growth factor (NGF)-induced neuritogenesis in PC12 cells, and its enhancing potency was attributed to the electrophilic center of 15d-PGJ₂. 15d-PGJ₂ indeed activated TRPV1, leading to a significant increase in the intracellular Ca^b level. In addition, the treatment of PC12 cells with biotinylated 15d-PGJ₂ resulted in the formation of a 15d-PGJ₂-TRPV1 adduct, indicating that 15d-PGJ₂ directly modified the TRPV1 in the cells. Furthermore, 15d-PGJ₂ facilitated the NGF-dependent signal transductions including ERK and JNK pathways in a Ca²⁺-dependent manner. These findings suggest that 15d-PGJ₂ enhances NGF signaling via TRPV1-dependent Ca^b influx, thereby acting as a potential neuritogenic enhancer in AD.

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Lipid peroxidation in Rheumatoid arthritis; consequences and monitoring

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Abstract

The epidemiological studies have shown that rheumatoid arthritis (RA) leads to oxidative stress formation. Therefore the aim of this study has been to estimate the lipid peroxidation in RA patients. Moreover the reasons and consequences of changes in lipid peroxidation were estimated. 73 patients with RA and 62 healthy subjects were included into the study. Lipid peroxidation was estimated by the measurement of phospholipid arachidonic acid (AA) and linoleic acid (LA) as well as aldehydes: 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal (4-HHE), malondialdehyde (MDA), acrolein, crotonaldehyde, and 4-oxononenal (4-ONE) that were determined by GC and GCMS, while 8-isoprostanes (8-isoPGF_{2α}) – was determined by LCMS. Phospholipase A₂ (PLA₂), platelet-activating factor acetylhydrolase (PAF-AH) and glutathione peroxidase (GSH-Px) activity were determined spectrophotometrically, while vitamin E was determined by HPLC. Plasma of RA patients was characterized by higher vitamin E level and decreased GSH-Px activity as well as the level of phospholipid AA and LA compared to healthy subjects. The level of all examined aldehydes was significantly increased in plasma patients but higher increase was observed in urine of RA patients in comparison with control group. The 8-isoprostanes level was approximately 9-fold higher in the plasma and 3-fold higher in the urine of RA patients compared to healthy subjects, but in the urine the changes in 8-isoprostanes level was correlated with RA progression. A significant increase in 4-HNE-modified protein adducts level as well as a decrease in the activity of PAF-AH and PLA₂ were observed in the plasma of RA patients. The level of lipid peroxidation products in the plasma and the urine may be the indicator of RA, but additionally urine 8-isoprostanes may be the useful and reliable biomarker of RA progression.

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