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Increased plasma xanthine oxidase activity is related to nuclear factor kappa beta activation and inflammatory markers in familial combined hyperlipidemia

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Abstract *Background and aims:* Xanthine oxidase (XO) has been described as one of the major enzymes producing free radicals in blood. Oxidative stress and inflammatory processes have been implicated in the pathogenesis of endothelial dysfunction and the progression of atherosclerosis but until now, there is little data about the influence of vascular prooxidant systems and inflammation in familial combined hyperlipidemia (FCH). Our goal was to evaluate whether XO activity was altered in FCH and if it was related to the inflammatory process represented by NFkB, IL-6 and hsCRP, and assessing the correlation between XO activity and insulin resistance (IR).
Method and results: 40 Non-related subjects with FCH and 30 control subjects were included, all of them non-diabetic, normotensive and non-smokers. We measured lipid profile, glucose, insulin, uric acid, XO activity, malondialdehyde (MDA), IL-6 and hsCRP in plasma and NFkB activity in circulating mononuclear cells. Patients with FCH showed significantly higher levels of uric acid, XO activity, MDA, NFkB activity, IL-6 and hsCRP than controls. XO activity was

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independently related to NFkB activity with an odds ratio of 4.082; to IL-6 with an odds ratio of 4.191; and to IR with an odds ratio of 3.830. Furthermore, mean NFkB activity, IL-6 levels, and IR were highest in the highest percentile of XO activity.
Conclusions: Subjects with FCH showed increased XO and NFkB activities and low grade inflammatory markers related to atherosclerosis. XO activity was correlated with higher inflammatory activity and IR. These data could explain, in part, the high cardiovascular disease risk present in these patients.

Introduction

It has been shown that classical coronary risk factors like hypercholesterolemia, smoking habits, hypertension and diabetes, activate oxidative systems in the vessel wall [1]. Moreover, in the last few years, oxidative stress and inflammatory processes have been implicated in the pathogenesis of endothelial dysfunction and progression of atherosclerosis [2,3], and they can explain how these classical factors can contribute to the pathogenesis of atherosclerosis.

Oxidative stress describes an imbalance between the production and degradation of reactive oxygen species (ROS). Therefore, an increase of the former activities and ROS production overwhelming the capacity of antioxidant enzymes leads to the establishment of oxidative stress, which contributes to endothelial dysfunction. Moreover, oxidative stress promotes an inflammation process, and inflammation is associated with oxidative stress that, in turn, maintains the atherogenic process [4].

Many of the enzymes implicated in the oxidative stress process are expressed in the vessel wall. These enzymes contribute to the production of ROS [1], which enhance activation of the transcription factor nuclear factor kappa beta (NFkB) that constitutes one of the key regulators of inflammation and oxidative stress, which controls the transcription of many genes with an established role in atherosclerosis [5]. One of the most important oxidative systems localized in the vessel walls is xanthine oxidase (XO) [6,7]. It is the terminal enzyme of purine catabolism that catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid (UA) and has been implicated in atherosclerosis and cardiovascular disease development [8,9].

Familial combined hyperlipidemia (FCH), one of the most common inherited disorders of lipid metabolism, is associated with an increased risk of atherosclerosis that is not fully explained by the metabolic disturbances of these patients [10]. We have recently shown that oxidative stress markers are increased in FCH, especially in the presence of insulin resistance (IR) [11], but up until now there is little data about the influence of vascular prooxidant systems and inflammation in FCH. Therefore, our objective was to evaluate whether XO activity was altered in FCH (and serum UA as an indirect marker of XO activity), and if it was related to malondialdehyde (MDA), as a marker of lipid peroxidation, and to the inflammation process, represented by NFkB activity, as a central regulator of the inflammatory process; IL-6 and hsCRP, as markers of inflammation, which in turn could be involved in premature atherosclerosis, assessing the possible correlation between XO activity and insulin resistance, in basal conditions and in the absence of pharmacological treatment.

Methods

Subjects

We studied 40 non-related subjects with FCH (15 women), selected by consecutive sampling at the Lipids Unit of our centre, and 30 control subjects (15 women); all subjects were non-diabetics, non-hypertensive, non-smokers, without clinical manifestations of cardiovascular disease and off treatment; their BMI was $<35\ kg/m^2$ and the age range between 18 and 65 years.

The inclusion criteria for the control group were: concentration of plasma total cholesterol (TC) <5.2 mmol/L, triglycerides (TG) <1.69 mmol/L, and apo B <1.2 g/L; fasting plasma glucose <5.5 mmol/L and the absence of a personal or familial history of dyslipidemia, cardiovascular disease or diabetes.

The diagnosis of FCH was based on the presence of hyperlipidemia (cholesterol and/or triglycerides concentration above the 90th percentile for our population by age and sex) and plasma apo B >1.2 g/L in the index patient, together with variable phenotypes IIa, IIb or IV in first degree relatives, a family history of arteriosclerosis and absence of xanthomas in the patient and in first degree family members [12].

Exclusion criteria were clinical manifestations of cardiovascular disease, diabetes, hypertension, smoking habit or smoker in the previous year, consumption of >30 g alcohol/day, intense physical fitness or weight-loss programs, body-weight fluctuation $>10\%$ in the previous three months, other chronic diseases, other secondary hyperlipidemias, renal or hepatic insufficiency and hypothyroidism, use of drugs capable of modifying the lipid profile, oxidative stress or inflammation that could not be withdrawn 6 weeks before initiating the study; and any infection, allergy or inflammatory disease in the six weeks prior to the study.

The study was approved by the ethical committee of our hospital and the subjects gave their informed consent.

Clinical and anthropometric parameters

Blood pressure was measured in the sitting position with a mercury sphygmomanometer after a 10-min period of rest, with two separated measurements; body mass index