Research Article

JOURNAL OF HEPATOLOGY

Xanthine oxidase in non-alcoholic fatty liver disease and hyperuricemia: One stone hits two birds

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Background & Aims: Hyperuricemia is a common feature of patients with non-alcoholic fatty liver disease (NAFLD). This study aimed to explore the causal relationship and underlying mechanisms between NAFLD and hyperuricemia.

Methods: We evaluated the impact of NAFLD on the development of hyperuricemia in a cohort of 5541 baseline hyperuricemia-free individuals. We further analyzed xanthine oxidase (XO), a rate-limiting enzyme that catalyzes uric acid production, as a candidate to link NAFLD and hyperuricemia.

Results: In the first study, a 7-year prospective analysis found that NAFLD was strongly associated with subsequent development of hyperuricemia. Cox proportional hazards regression analyses showed that age, gender, and body mass index adjusted hazard ratio (95% confidence interval) for incident hyperuricemia was 1.609 (1.129–2.294) in individuals with NAFLD, as compared with those without NAFLD at baseline. In the second study, we observed that expression and activity of XO were significantly increased in cellular and mouse models of NAFLD. Knocking down XO expression or inhibiting XO activity significantly decreases uric acid production and attenuates free fatty acids-induced fat accumulation in HepG2 cells. Inhibiting XO activity also significantly prevents the development of and ameliorates established hepatic steatosis induced by a high-fat diet in mice. Further experiments indicated that XO regulates activation of the NLRP3 inflammasome, which may be essential for the regulatory effect of XO on NAFLD.

Conclusions: NAFLD significantly increases the risk of incident hyperuricemia. XO is a mediator of the relationship between NAFLD and hyperuricemia, and may serve as a novel therapeutic target for the two linked diseases.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide [1]. The disease affects 15% to 20% of adults in China [2], and approximately one-third of adults in the United States [3]. NAFLD comprises a broad spectrum of disease stages, including simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis [4,5]. Simple steatosis is considered to be benign with a slow progression over years, whereas NASH may progress to cirrhosis and hepatocellular carcinoma [6]. Recently, NASH has been ranked as the second leading etiology of hepatocellular carcinoma requiring liver transplantation in the United States [7].

NAFLD is closely associated with obesity and related metabolic disorders [8,9]. Hyperuricemia or high serum uric acid levels are common metabolic abnormalities occurring in obese individuals [10]. In our previous study, we reported that serum uric acid levels are significantly elevated in NAFLD patients [11]. Subsequent epidemiological studies in different populations confirmed a significant association between serum uric acid and NAFLD [12–14]. We and other groups also found that the elevation of serum uric acid in healthy individuals independently predicts an increased incidence of NAFLD [15–17]. However, whether the vice versa is true, that is, whether NAFLD increases the risk for subsequent development of hyperuricemia remains

Keywords: Fatty liver; Hyperuricemia; Uric acid; Xanthine oxidase; NLRP3 inflammasome.

Received 5 June 2014; received in revised form 24 November 2014; accepted 14 January 2015; available online 23 January 2015

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Abbreviations: CI, confidence interval; COX-2, cyclooxygenase-2; DMEM, Dulbecco's Modified Eagle Medium; FFA, free fatty acids; H&E, hematoxylin-eosin; HFD, high-fat diet; HR, Hazard ratios; IL-1β, interleukin-1β; MCD, methionine and choline deficient; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NLRP3, NOD-like receptor family pyrin domain containing 3; ROS, reactive oxygen species; PPARγ, peroxisome proliferator-activated receptor gamma; RT-PCR, reverse transcription polymerase chain reaction; SCD, standard chow diet; siRNA, small interfering RNA; XO, xanthine oxidase.

unclear. Furthermore, the molecular mechanisms behind the association between NAFLD and hyperuricemia are not known.

Xanthine oxidase (XO), also known as xanthine oxidoreductase, is a rate-limiting enzyme that catalyzes uric acid production with concomitant generation of reactive oxygen species (ROS) [18]. The enzyme catalyzes the final two steps in purine catabolism, modulating hypoxanthine to xanthine and then to uric acid [19]. Expression of XO is highest in liver and intestine [20], suggesting a potential organ-specific physiological function of the enzyme. XO has recently been observed to be involved in the regulation of lipogenesis and atherosclerosis [21,22]. To date, the role of XO in NAFLD has not been well elucidated. Whether XO contributes to the relationship between NAFLD and hyperuricemia is unknown.

NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a protein complex that integrates interleukin (IL)-1β and IL-18 processing to sense cells for pathogen- and danger-associated signals [23,24]. Uric acid and ROS are two common activators of NLRP3 inflammasome [25,26]. NLRP3 inflammasome activation participates in the pathogenesis of obesity and insulin resistance [27,28]. A significant contribution of NLRP3 inflammasome activation to the development of NAFLD has been shown by recent studies [29,30]. To the best of our knowledge, a detailed regulatory mechanism for NLRP3 inflammasome activation in NAFLD has not been described yet. XO is a key enzyme that catalyzes the production of uric acid and ROS, whether XO is involved in the regulation of NLRP3 inflammasome activation has not yet been examined.

In this study, we firstly investigated the impact of NAFLD on the development of hyperuricemia in a 7-year longitudinal study, and then analyzed the involvement of XO in the association between NAFLD and hyperuricemia.

Materials and methods

The two linked studies reported herein were designed to clarify the causal relationship between NAFLD and hyperuricemia, and to reveal potential underlying mechanisms for such association. The first one is a population-based longitudinal study aimed to explore the impact of NAFLD on development of hyperuricemia. After having collected the results of the first study, we then sought to identify a molecular explanation for the association between NAFLD and hyperuricemia. To this end, we analyzed the involvement of XO in the relationship between NAFLD and hyperuricemia, and investigated whether modulating XO expression and activity have therapeutic benefits for the two linked diseases. Details of the methods are described in Supplementary Materials and methods.

Results

The first study

Baseline characteristics and follow-up outcomes

Of the 5541 participants (3355 men and 2186 women, with a mean age of 41.1 ± 12.3 years and 43.6 ± 10.4 years, respectively) enrolled at baseline, 4557 completed the 7-year follow-up assessment, and another 892 completed at least one follow-up assessment during the 7-year period, while the remaining 92 participants did not attend any follow-up assessment. Clinical characteristics were not statistically different between participants who successfully followed-up and those who did not complete any follow-up assessment (Supplementary Table 1).

Of 5449 participants successfully followed-up, NAFLD was identified in 281 at baseline. Baseline characteristics of the study participants according to NAFLD status are presented in Table 1. Although all the participants were metabolically healthy at baseline, participants with NAFLD showed more unfavorable

Table 1. Comparison of clinical characteristics according to baseline NAFLD status.

	Without NAFLD	With NAFLD	*t* value	*p* value
n (male/female)	5168 (3055/2113)	281 (231/50)	59.370[a]	<0.001
Age (years)	42.0 (11.6)	44.1 (11.9)	3.009	0.003
Body mass index (kg/m^2)	21.64 (2.42)	25.02 (2.14)	22.960	<0.001
Waist circumference (cm)	74.3 (7.2)	84.6 (6.0)	23.560	<0.001
Systolic blood pressure (mmHg)	115.4 (12.9)	121.2 (11.3)	7.395	<0.001
Diastolic blood pressure (mmHg)	73.2 (8.2)	76.6 (7.6)	6.742	<0.001
Alanine aminotransferase (U/L)	19.0 (14.0-27.0)	39.0 (26.0-59.0)	17.560[b]	<0.001
Aspartate aminotransferase (U/L)	19.0 (16.0-22.0)	24.0 (19.0-29.0)	12.246[b]	<0.001
γ-Glutamyltransferase (U/L)	15.0 (11.0-21.0)	30.0 (20.0-43.0)	16.469[b]	<0.001
Triglyceride (mmol/L)	1.01 (0.78-1.34)	1.42 (1.14-2.03)	13.793[b]	<0.001
Total cholesterol (mmol/L)	4.71 (0.90)	5.16 (0.90)	7.977	<0.001
HDL-cholesterol (mmol/L)	1.35 (1.12-1.64)	1.24 (1.10-1.49)	4.386[b]	0.004
LDL-cholesterol (mmol/L)	2.62 (0.74)	3.05 (0.74)	9.335	<0.001
Fasting serum glucose (mmol/L)	4.38 (4.11-4.69)	4.52 (4.20-4.87)	3.989[b]	<0.001
Serum uric acid (μmol/L)	292.6 (66.1)	336.7 (53.1)	10.985	<0.001
Serum creatinine (mg/dl)	69.0 (58.0-79.0)	73.0 (65.0-80.0)	3.753[b]	<0.001
Blood urea nitrogen (mmol/L)	4.88 (4.14-5.72)	5.08 (4.30-6.00)	2.656[b]	0.008
White blood cell count (×10^9/L)	6.0 (1.5)	6.6 (1.5)	6.699	0.009
Erythrocyte sedimentation rate (mm/h)	7.0 (4.0-10.0)	7.0 (3.0-10.0)	1.668[b]	0.095

Data are expressed as means (SD) or medians (IQR) depending on the data distribution.
HDL, High-density lipoprotein; LDL, low-density lipoprotein.
[a]χ^2 value; [b]Z value.