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Inhibition of the JAK/STAT pathway by ruxolitinib ameliorates thioacetamide-induced hepatotoxicity

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ABSTRACT

In an attempt to explore the role of the JAK/STAT pathway in liver inflammation, we investigated the effect of intervening this pathway by ruxolitinib in thioacetamide (TAA)-induced hepatotoxicity. Ruxolitinib treatments were administered to male mice either before or after intoxication with TAA. The hepatic histopathological and serum biochemical assessment revealed that ruxolitinib pre-treatments dose-dependently reduced TAA-induced liver injury, caspase 3 cleavage and increase in number of hepatocytes positive for the pro-apoptotic Bax, as well as inflammatory cells positive for F4/80 and myeloperoxidase activity in the liver. Ruxolitinib pre-treatments also curbed TAA-induced rise in NF-κB nuclear expression and STAT3 phosphorylation. Ruxolitinib pre-treatments also lowered TAA-induced elevation of hepatic oxidative stress parameters (total nitrate/nitrite and 4-hydroxynonenal), but did not restore the hepatic antioxidant reduced glutathione. Interestingly, ruxolitinib, especially at a dose of 200 mg/kg, dampened the overproduction of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-23 and IL-17A), which coincided with boosting the release of the anti-inflammatory cytokine IL-10. Ruxolitinib when used as a post-treatment (1 and 3 h after TAA-insult) could still spare the liver from injury and might be clinically applicable. In conclusion, the multimechanistic-hepatoprotective activity of ruxolitinib can be linked to its ameliorative properties on cellular death, oxidative stress and inflammation machinery.

1. Introduction

Hepatic diseases are a major cause of worldwide morbidity and mortality. Most types of hepatic diseases are characterized by inflammatory processes with enhanced expression of various pro-inflammatory cytokines in the liver. One of the major pathways involved in the signal transductions of wide arrays of these cytokines is the Janus kinase/signal transducers and activators of transcription (JAK/STAT) cascade. The JAK/STAT pathway is a key player in many important biological processes, including broad immune and hematopoietic cell functions (Rane and Reddy, 2000). On the other hand, erratic function of this pathway is widely implicated in various types of illness, such as autoimmune diseases, hematopoietic disorders, graft rejection and inflammation, (Ghoreschi et al., 2011).

JAKs comprise a group of 4 tyrosine kinases (JAK1, JAK2, JAK3 and TYK2) that selectively associate with cytokine receptor chains and transduce signaling through phosphorylating tyrosine residues on themselves and STATs (Pesu et al., 2008). STATs subsequently become dimerized and transport to the nucleus, where they activate or suppress the gene transcription (Harrison, 2012). Thus, the pharmacological modulation of elements of this pathway may represent a novel treatment approach for inflammatory and immune-mediated diseases.

Ruxolitinib, a novel oral JAKs 1 and 2 inhibitor, was recently approved as a revolutionary therapy for patients suffering from intermediate/high risk myelofibrosis (Mascarenhas and Hoffman, 2012). Although the efficacy of ruxolitinib in myelofibrosis is now well established, data about the effect of ruxolitinib in inflammatory disorders, especially those occurring in the liver, are still limited. Most recently, we found that pre-treatment with ruxolitinib protected mice from carbon tetrachloride-induced hepatotoxicity (Hazem et al., 2014). In this study, we examined whether the capability of ruxolitinib to confer hepatoprotection is also extended to thioacetamide (TAA)-induced hepatotoxicity

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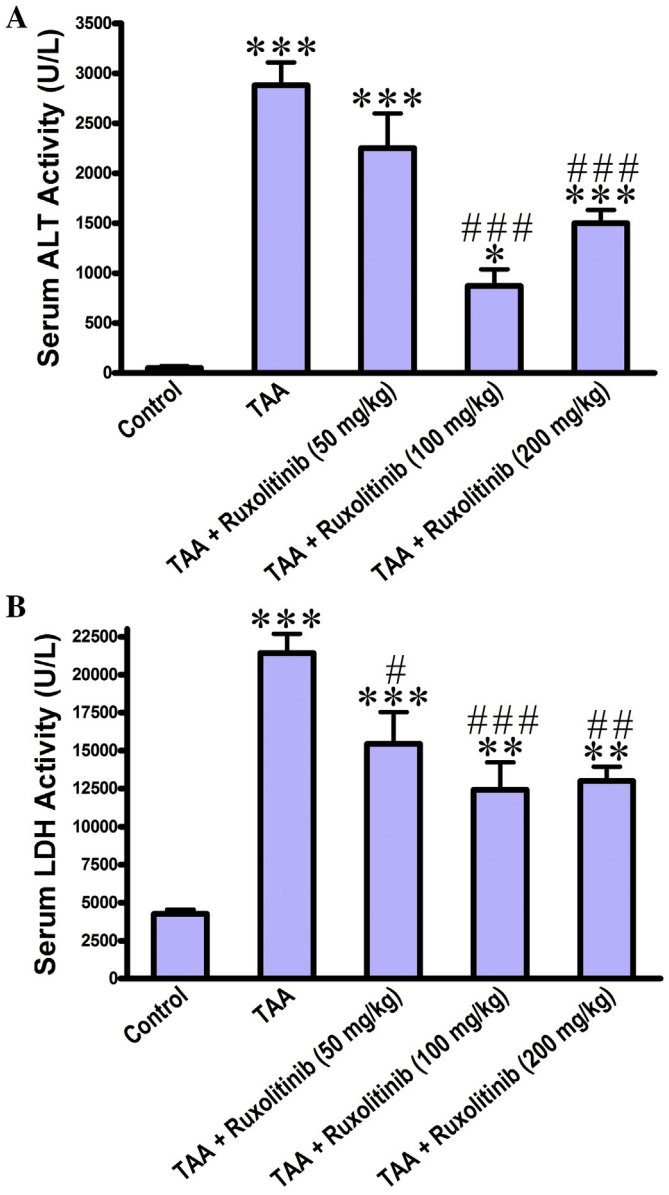

Fig. 1. Effects of ruxolitinib (50, 100 and 200 mg/kg) pre-treatments on serum alanine aminotransferase (ALT, A) and lactate dehydrogenase (LDH, B) activities in thioacetamide (TAA)-induced acute liver injury. Bars are means ± SE (n = 7). *, ** and *** denote statistical significance from Control group at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, while #, ## and ### denote statistical significance from TAA group at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

mouse model. Additionally, we tested administering ruxolitinib at different time-points from TAA intoxication to evaluate the post-treatment strategy and simulate the clinical application in humans.

2. Materials and methods

2.1. Animals

Male adult BALB/c mice (33–37 g) had free access to tap water and diet and acclimatized at least 1 week prior experiments. All the mice included in this study received care according to the guidelines of NIH and Research Ethics Committee, Faculty of Pharmacy, University of Mansoura.

2.2. Experimental design and administration of drugs and chemicals

Ruxolitinib in the form of phosphate salt was supplied as a generous gift form Novartis Pharmaceuticals (Basel, Switzerland). Different concentration of ruxolitinib (0.5, 1 and 2% w/v) was freshly prepared in physiological-saline containing 0.5% (w/v) of carboxymethylcellulose, while TAA (Sigma, St. Louis, MO, USA) was prepared as 3% (w/v) in physiological saline. Based on these concentrations, the volumes of all administrations were adjusted to 350 μl/35 g mice.

In the pre-treatment experiments, different doses of ruxolitinib (50, 100 and 200 mg/kg) were given by oral route (p.o.) 2 h before intraperitoneal (i.p.) intoxication with one dose of TAA (300 mg/kg) for 30 h subsequent to overnight fasting. Another group administered the vehicle without ruxolitinib (p.o.) and TAA 2 h afterwards and served as TAA untreated mice. The last group served as a normal control mice, which were administered the vehicle without ruxolitinib (p.o.) and physiological saline without TAA (i.p.) 2 h afterwards. In another set of experiments, mice were initially challenged with TAA and post-treated with ruxolitinib (100 mg/kg, p.o.) at 1, 3 and 6 h afterwards to simulate the clinical application in hepatic inflammatory disorders.

After 30 h from TAA challenge, mice were anesthetized and blood samples were collected from the heart. Blood samples were then subjected to centrifugation at 2000*g* for 10 min at 4 °C for separation of serum, followed by storage at −80 °C. Some pieces of liver tissues were kept at −80 °C for the antioxidant and oxidative stress assays, as well as Enzyme-linked immunosorbent assay (ELISA). Also, some pieces of liver tissues were kept in normal saline solution containing 10% (v/v) of formaldehyde for histopathology and immunohistochemistry. Some mice were also sacrificed after 12 h from TAA injection, and livers were collected for protein extraction, electrophoresis and Western blotting analysis.

2.3. Biochemical parameters of hepatocellular injury

The enzymatic activities of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were estimated in serum by kinetic kits purchased from Spectrum Diagnostics (Cairo, Egypt).

2.4. Liver histopathology and immunohistochemistry

Routine protocols of histopathology were used for processing fixed liver pieces into paraffin blocks, cutting the liver sections (5 μm thick) and staining with hematoxylin-eosin (H-E). Scoring of necroinflammation injury mediated by TAA was as follows: a score of 0 in case of absence of necrosis; a score of 1 in case of spotty necrosis (one or few necrotic hepatocytes); a score of 2 in case of confluent necrosis; and a score of 3 in case of bridging necrosis (Gonzalez-Periz et al., 2006). Liver specimens were also evaluated immunohistochemically for the protein expression of nuclear factor-kappa B (NF-κB) p65, Bcl2-associated X protein (Bax) and F4/80 using primary antibodies (diluted 1:250) purchased from Bio-Legend (San Diego, CA, USA).

2.5. Enzyme-linked immunosorbent assay (ELISA) for cytokines

Liver pieces were homogenized (10% w/v) in cold lysis buffer (150 mM NaCl, 0.5% v/v Triton X-100, 10 mM Tris pH 7.4) supplemented with 2 mM of the protease inhibitor 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (Acros Organics, Morris Plains, NJ, USA). Following lysis on ice for 30 min, the liver samples were subjected to centrifugation at 8000*g* for 10 min at 4 °C, followed by collecting the supernatants for ELISA. Mouse interferon-γ