Inhibitory effect of oxymatrine on hepatocyte apoptosis *via* TLR4/ PI3K/Akt/GSK-3β signaling pathway

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1. What did this study explore?

In this study, we explored the therapeutic effect of OMT on lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced ALF in rats, thus exploring whether OMT can inhibit hepatocyte apoptosis via the TLR4/PI3K/Akt/GSK-3β signaling pathway.

2. How did the authors perform all experiments?

The experiments were conducted in the laboratory of pathophysiology of Nantong University. Animals were adapted to the environment for 1 week. One hundred male Sprague–Dawley rats weighing 220–250 g were randomly assigned to five groups. Rats were anesthetized with ketamine and killed by decapitation at 24 h after LPS/GalN injection. Blood and liver samples were collected for further assessment. Pathological examination of the liver was conducted using hematoxylin and eosin (HE) staining. Transmission electron microscopy (TEM) was adopted to observe morphological changes of apoptosis. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by biochemical method using automatic biochemical analyzer (Olympus, Japan). This test was measured in the Department of Biochemistry at the Affiliated Hospital of Nantong University. We detected expression of tumor necrosis factor (TNF)-α and

interleukin (IL)-1β using enzyme-linked immunosorbent assay (ELISA). Flow cytometry were used to detected the apoptotic rate of hepatocytes. The protein expression was evaluated by Western blotting method and immunohistochemistry.

3 How did the authors process all experimental data?

The results were analyzed with SPSS version 18.0. Data are presented as mean ± SEM. Statistical significance of differences between groups was evaluated by one-way analysis of variance with Tukey's multiple comparison test. $P < 0.05$ was considered statistically significant. Image J was used to evaluate the gray level of each protein. Pathological examination of the liver was conducted by two experienced pathologists. Consensus was obtained between two pathologists.

4 How did the authors deal with the pre-study hypothesis?

We have previously confirmed that the TLR4/PI3K/Akt/GSK-3β pathway participates in the regulation of apoptosis in BRL-3A cells. we supposed the therapeutic effect of OMT on lipopolysaccharide (LPS)/D-galactosamine (D-GalN)-induced ALF in rats, thus exploring whether OMT can inhibit hepatocyte apoptosis via the TLR4/PI3K/Akt/GSK-3β signaling pathway.

5 What are the novel findings of this study?

We are the first to investigate the role of OMT in the inhibition of apoptosis in acute liver failure and its potential mechanisms.