Effect of hepatocyte apoptosis induced by TNF-α on acute severe hepatitis in mouse models

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Abstract

AIM To study the effect of hepatocyte apoptosis and necrosis induced by TNF-α on the pathogenesis of acute severe hepatitis (ASH).

METHODS The model of ASH was prepared in D-galactosamine (GalN) sensitized BALB/c mice by injection of either endotoxin (ET) or tumor necrosis factor-α (TNF-α). Morphological changes of apoptotic hepatocytes were studied by both light and electron microscope and in situ end labeling method (ISEL). Molecular biological changes of DNA ladder were observed by electrophoresis of extract from liver tissues. Biochemical changes were measured by alanine aminotransferase (ALT), aspartate transaminase; endotoxins; mice.

CONCLUSION TNF-α can cause liver damage by inducing hepatic apoptosis and necrosis in mice with endotoxemia.

INTRODUCTION

Apoptosis is one of the cell death forms, which is quite different from cell necrosis in morphology, biochemistry and biology. Clinical studies showed that ET (Endotoxin, ET) and tumor necrosis factor-α (TNF-α) elevated obviously in the sera of patients with severe hepatitis. Recent researches showed that hepatocyte apoptosis was closely related with pathogenesis of hepatitis especially that of ASH[1-11]. The present study deals with the effect of both apoptosis and necrosis induced by TNF-α on the pathogenesis in ASH, and their relationship.

MATERIAL AND METHODS

Reagents

Recombinant murine TNF-α and immunoglobulin (Ig) G1 fraction of anti-murine TNF-α were purchased from Pepro Tech EC LTD. Terminal-deoxynucleotidyl transferase (TdT) and Bio-11-dUTP Na salt were purchased from Dako LTD. Salmonella abortus equi endotoxin (ET) was purchased from Sigma Chemical Co. GalN was purchased from Chong Qing Medical University. Endogenous mouse TNF-α ELISA Kit was provided by Endogen Inc. Unless otherwise specified, all other reagents were analytical reagents.

Animal and experimental methods

One hundred and thirty specific pathogen-free male BALB/c mice (from Shanghai Second Medical university animal breeding house) were divided into 7 groups: 1. Control; 2. GalN; 3. ET; 4. TNF-α; 5. GalN/ET; 6. GalN/TNF-α; and 7. anti-TNF-α IgG1/GalN/ET.

Each group contained 20 mice except Group 7 which contained 10 mice. Both GalN (800mg/kg) and ET (2.4µg/kg) were injected intraperitoneally; Both TNF-α (1.0µg/kg) and anti-TNF-α IgG1 (100µg/mouse) were injected in tail vein. The control group received the same volume of normal saline.

Five mice were killed at 1.5, 3.5, 6, and 9 h respectively after injection. TNF-α, ALT, and AST were measured in the blood samples taken from the mouse heart. Tissue samples taken from liver were also prepared for morphological and molecular biological examinations.

The sections of liver tissue were stained with hematoxylin and eosin (HE), and ISEL[12] for detection of apoptotic liver cells were performed. DNA was extracted from fresh liver tissue for further analysis of DNA ladder.
RESULTS

Histological alterations of the liver in control, GalN, ET, and TNF-α groups

Hepatic lobular architecture was clear and intact without any abnormalities in the liver section of control group. Only mild swelling of hepatic cell was presented in GalN, ET and TNF-α groups. With the time prolonging from 1.5 h, 3.5 h, 6 h, to 9 h, the mild swelling become moderate degree. Neither apoptosis nor necrosis was present in HE and ISEL staining. No DNA ladder was found in any group mentioned above.

Histological alterations of the liver in GalN/ET group

No obvious liver cell alterations were present at 1.5h on the section of HE staining and ISEL staining failed to detect positive signals of apoptosis.

Mild swelling of liver cells on HE section and a few apoptotic cells on ISEL section were present at 3.5h (Figure 1A).

Obvious swelling of liver cells and lots of apoptotic cells were found at 6h and mild dotted necrosis was also observed. Strong apoptotic positive signals could be detected in the cell nuclei.

Nine hours after GalN/ET injection, enormous apoptotic liver cells and pieces of hepatic necrosis with leukocytes infiltration can be seen (Figure 1B).

These data demonstrate that apoptosis occurred as an early event during the development of hepatic failure. Compared with the ET model, the majority of changes occurred earlier in the TNF model, which agrees with the phenomena that ET act on hepatocytes by inducing TNF-α.

Histological alterations of the liver in GalN/TNF-α group

Morphological changes of the liver in this group were basically the same as those in GalN/ET group but severer at 1.5 h, 3.5 h, 6 h and 9 h (Figures 2A, 2B), 1.5 h, 3 h, 6 h and 9 h after administrating inducer, apoptosis positive rates of GalN/ET group were 0.0%, 0.2%, 1.21% and 3.14% respectively, while for the GalN/TNF-α group, were 0.2%, 0.5%, 2.57% and 3.19% respectively.

DNA fragment assay

DNA ladder was found at 6 h and 9 h after GalN/ET, GalN/TNF-α administration, but was not found at 1.5h and 3.5h after GalN/ET, GalN/TNF-α injection, and DNA ladder was not found in other groups (Figures 3A,3B).

Electron microscopic assay

Electron microscopic study showed the chromatin condensation of apoptotic cells and near the nuclear lining (Figure 4).

Blockage of liver damage induced by GalN/ET by pretreatment of mice with anti-TNF-α IgG1

In the GalN/ET group pretreated with anti-TNF-α IgG1, only mild swelling of liver cells could be seen on the HE staining. Neither apoptosis nor necrosis could be found on ISEL staining. No DNA ladder was present on electrophoresis of agarose gel (Figure 3A).

Time course of apoptosis and necrosis

We compared the time course of typical morphology of apoptosis (chromatin condensation, apoptotic bodies) with ALT and AST (as a parameter for necrosis) in each group (Table 1). ALT and AST remained normal at 3.5h after GalN/TNF-α challenge, at this time point, a small amount of apoptotic liver cells could be found. Apoptotic liver cells become obvious at 6h, ALT and AST were elevated mildly at the same time. This finding indicated that apoptosis was already developed while the liver cell membrane still remained intact 6 h after challenge, suggesting that apoptosis occurred earlier than necrosis.

The prominent increase of ALT and AST occurred at 9h, when a great number of necrotic liver cells were observed. Meanwhile, profuse apoptotic liver cells were also present even after the death of mice associated with ASH and electrophoresis of agarose gel still showed DNA ladder at the final stage.

Figure 1  Liver cells apoptosis in mice induced by GalN/ET. A 3.5 hours after GalN/ET, individual apoptotic cells are visible, apoptotic positive signal mainly locates in nucleus. ISEL×400 B 9 hours after GalN/ET, further increase of apoptotic liver cells and pieces of liver cell necrosis and bleeding with leukocytes infiltration appear. Apoptotic cells (a), apoptotic bodies (b), leukocytes (1) and necrosis (n). HE×400
DISCUSSION

ASH may be caused by viral infection and drug intoxication. It was believed that the large amount of liver cell death was necrosis due to associated immune damage mediated by dysfunction of host immune system and TNF-α may cause liver necrosis directly\(^{13,14}\). Recent studies have shown that besides necrosis, hepatic apoptosis induced by TNF-α plays an important role in the course of ASH\(^{1,15-27}\). Our study showed that only mild injury could be found by injecting ET or TNF-α alone. While the combination of GalN with either ET or TNF-α can cause ASH in mice.

Liver cells may synthesize the protecting protein after exposure to injury factors. The process needs the participation of intact cyto-metabolism and protein-synthesis mechanism. GalN may specifically deplete uridine nucleotides in liver cell and influence its metabolic course, leading to a hepatic transcriptional block and the suppression of

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**Table 1** Comparison of TNF-α, ALT and AST at corresponding time in different groups (\(n = 5, \bar{x} \pm s\))

|        | 1 h  | 3 h  | 6 h  | 9 h  |
|--------|------|------|------|------|
| TNF    | 13.9±15.0 | 39.6±10.3 | 124.0±17.0 | 18.8±15.2 |
| ALT    | 18.8±15.2 | 40.1±6.0   | 129.6±33.5 | 28.0±21.1  |
| AST    | 10.4±16.2 | 40.8±12.8  | 118.4±40.1 | 45.2±35.7  |
|        | 15.9±15.9 | 41.5±5.4   | 100.1±24.3 | 25.4±29.7  |
|        | 20.0±31.1 | 46.8±4.2   | 135.4±62.4 | 47.3±9.0   |
|        | 28.0±43.3 | 47.2±23.5  | 63.0±20.1  | 100.2±35.5 |
|        | 50.8±51.3 | 52.4±23.0  | 194.3±62.4 | 58.2±30.0  |
|        | 123.7±84.3 | 223.0±85.0 | 150.1±17.8 | 51.1±25.4  |
|        | 147.3±103.9 | 230.1±82.9 | 127.3±70.1 | 119.5±98.4 |
|        | 32.1±14.6 | 89.1±37.1  | 325.3±138.4 | 230.1±82.9 |
|        | 33.7±18.0 | 46.0±13.4  | 108.6±36.0 | 268.3±380.8 |
|        | 36.3±27.0 | 48.4±14.7  | 129.8±50.3 | 798.2±319.8 |

Comparing of results with controls at the corresponding time: \(p < 0.05\)
Comparing of each other group at the different time. TNF-α: \(p < 0.05\); ALT: \(p < 0.05\); AST: \(p < 0.05\).
TNF-α may induce apoptosis of liver cell which is transferred by hepatitis B virus or other virus[35-41], suggesting the cells infected by virus involved in TNF-α sensitivity.

The results of our study showed that TNF-α was mainly produced in the early stage of endotoxemia, and decreased obviously from 6h to 9h after challenge. TNF-α combined with TNF-α receptor on the membrane of liver cells through a series signal transmission activating caspase-3 and then inducing apoptosis, and TGF-β1 can also produce similar effect which can induce apoptosis[42-45], delayed treatment with the caspase 3-like protease inhibitor Z-VAD attenuated apoptosis by 81% to 88% and prevented liver cell necrosis[46]. At the same time TNF-α can activate nuclear transription factor-κκ (NF-κκκ) of hepatocytes[47], Kuppfer cells and endotheliocyte, which increases expression of ICAM-1, VCAM-1 and selectin, these inflammatory factors further induce the inflammatory injury of hepatocytes, and TNF-α also induce Shwartzman-like reaction in the liver[48]. Recent study demonstrated that mitochondria may be the centre of cell apoptosis, if mitochondrial structural alterations occur without functional failure, the cell dies by apoptosis. In contrast, if the injury is severe enough to lead to mitochondrial functional failure, the cell dies by necrosis[49,50].

In summary, our results showed that TNF-α plays an important role in the course of hepatic apoptosis and necrosis. The blockage of liver apoptotic signal transmission and caspase activation induced by TNF-α with Z-VAD, anti-ET antibody and anti-TNF monoclon antibody can improve prognosis of fulminant hepatic failure[2,42-46,51] and may prevent liver cell from apoptosis and necrosis and hence has an important significance in the prevention and treatment of ASH.

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