Glycyrrhizin attenuates HMGB1-induced hepatocyte apoptosis by inhibiting the p38-dependent mitochondrial pathway

Geum-Youn Gwak, Tae Gun Moon, Dong Ho Lee, Byung Chul Yoo

AIM: To examine how high-mobility group box 1 (HMGB1) regulates hepatocyte apoptosis and, furthermore, to determine whether glycyrrhizin (GL), a known HMGB1 inhibitor, prevents HMGB1-induced hepatocyte apoptosis.

METHODS: A human hepatocellular carcinoma cell line stably transfected with a bile acid transporter (Huh-BAT cells), were used in this study. Apoptosis was quantified using 4',6-diamidino-2-phenylindole dihydrochloride staining and the APO Percentage apoptosis assay, and its signaling cascades were explored by immunoblot analysis. Kinase signaling was evaluated by immunoblotting and by using selective inhibitors. It is also tried to identify hepatocyte apoptosis affected by the HMGB1 inhibitor, GL.

RESULTS: HMGB1 increased cellular apoptosis in Huh-BAT cells. HMGB1 led to increased cytochrome c release from mitochondria into the cytosol, and induced the cleavage of procaspase 3. However, it did not affect the activation of caspase 8. HMGB1-induced caspase 3 activation was significantly attenuated by the p38 inhibitor SB203580. GL significantly attenuated HMGB1-induced hepatocyte apoptosis. GL also prevented HMGB1-induced cytochrome c release and p38 activation in Huh-BAT cells.

CONCLUSION: The present study demonstrated that HMGB1 promoted hepatocyte apoptosis through a p38-dependent mitochondrial pathway. In addition, GL had an anti-apoptotic effect on HMGB1-treated hepatocytes.

Key words: High-mobility group box 1; Hepatocyte; Apoptosis; Glycyrrhizin; p38; Mitochondria

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INTRODUCTION

High-mobility group box 1 (HMGB1) is an evolutionarily conserved protein present in the nucleus of almost all eukaryotic cells, where it is involved in DNA replication, repair and transcription. This molecule is known to be released by cells undergoing necrosis as well as being...
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secreted by activated macrophages. While early studies of HMGB1 demonstrated its role as a late mediator of sepsis,[9] HMGB1 has been more recently implicated as a putative danger signal involved in the pathogenesis of a variety of non-infectious inflammatory conditions including autoimmunity, cancer, trauma, and hemorrhagic shock, and ischemia-reperfusion injury (IRI).[10-12] So far, HMGB1 has been studied in a number of organs including liver, lung, breast and prostate.[5,9,11]

In the liver, the importance of HMGB1 signaling has been largely identified in cases of IRI, during which tissue levels of HMGB1 were elevated following reperfusion and neutralizing antibodies against HMGB1 ameliorated the damage resulting from IRI in a toll-like receptor (TLR)4-dependent manner.[13] The pathogenic role of HMGB1 in liver disease was also clarified by studying the inflammatory response to viral infection.[14] Following hepatocyte death by hepatitis B virus-specific cytotoxic T lymphocytes in a mouse model of hepatitis, HMGB1 directs the intrahepatic recruitment of neutrophils and all other non-antigen specific inflammatory cells (natural killer cells, T cells, B cells, monocytes, macrophages and dendritic cells).

Apoptosis, a stereotyped morphologic form of cell death, is an event that contributes to liver injury in a wide range of acute and chronic liver diseases.[15,16] However, it is not clear whether HMGB1 contributes to apoptotic cell death in the liver. Furthermore, the regulatory mechanism of HMGB1 in hepatocyte apoptosis remains largely undefined.

Glycyrrhizin (GL) is a major active constituent of licorice root that is commonly used in Asia to treat patients with chronic hepatitis.[17-19] This compound has been associated with numerous pharmacologic effects, including anti-inflammatory, anti-viral, anti-tumor, and hepatoprotective activities.[20,21] Recently, GL was recognized by Sitia et al.[22] as an HMGB1 inhibitor, which binds directly to both HMGB boxes in HMGB1.

Thus, the aim of this study was to provide evidence and a potential theoretical basis for HMGB1 regulation of hepatocyte apoptosis in order to further elucidate the molecular mechanism of HMGB1 involvement in various pathologic conditions that can affect the liver. Furthermore, we attempted to determine whether GL attenuates HMGB1-induced hepatocyte apoptosis and, if so, to identify the signaling cascades responsible for this modulation.

MATERIALS AND METHODS

Cell line and culture
Several human hepatoma cell lines were chosen for this study: Huh-7 cells stably transfected with a bile acid transporter[23] derived from a well-differentiated hepatocellular carcinoma (HCC)[24] (Huh-BAT), HepG2 and SNU-475 cells derived from a poorly differentiated HCC.[25] All cells were cultured in Dulbecco’s Modified Eagle medium supplemented with 10% fetal bovine serum, 100 000 U/L penicillin and 100 mg/L streptomycin. In all experiments, cells were serum-starved for 12 h in order to avoid the effects of serum-induced signaling.

Materials and reagents
HMGB1 (human, recombinant expressed in E. coli) was synthesized by Sigma-Aldrich, Inc. (St. Louis, MO, United States) at a purity of > 90%. The MAPK inhibitors [SB203580 for p38 mitogen activated protein kinase (MAPK), U0126 for p42/44 MAPK or extracellular signal-regulated kinase, and SP600125 for c-Jun N-terminal kinase (JNK) and GL] were also obtained from Sigma-Aldrich, Inc.

Preparation of mitochondrial and cytosolic extracts
Cells were washed twice with phosphate-buffered saline, and mitochondrial and cytosolic extracts were isolated using a mitochondria/cytosol fractionation kit (BioVision, Inc., Mountain View, CA, United States) according to the manufacturer’s instruction.

Immunoblot analysis
Huh-BAT cells were lysed for 20 min on ice with lysis buffer (50 mol/L Tris-HCl, pH 7.4; 1% Nonidet P-40; 0.25% sodium deoxycholate; 150 mol/L NaCl; 1 mol/L EDTA; 1 mol/L PMSF; 1 μg/mL aprotinin, leupeptin, pepstatin; 1 mol/L Na3VO4; and 1 mol/L NaF) and centrifuged at 14 000 × g for 10 min at 4 °C. Proteins in the lysates were resolved by 10% or 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis, transferred to PVDF membranes, and probed using the following primary antibodies: mouse anti-caspase 8 (1:500 dilution) from Cell Signaling Technology (Danvers, MA, United States); rabbit anti-caspase 3 (1:1000 dilution) from Cell Signaling Technology; rabbit anti-ACTIVE® p42/p44 (1:2000 dilution), anti-ACTIVE® p38 (1:1000 dilution), and anti-ACTIVE® JNK (1:1000 dilution) specific for the phosphorylated forms of p42/p44 MAPK, p38 MAPK, and JNK, respectively, from Cell Signaling Technology; mouse anti-cytochrome c (1:500 dilution) from BD Pharmingen (San Jose, CA, United States), and goat anti-actin (1:1000 dilution) from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, United States). Twenty μg of protein was used for each well in Western blotting. Primary antibody binding was detected with appropriate peroxidase-conjugated secondary antibodies (Biosource Technology Inc. (Santa Cruz, CA, United States). Bound antibodies were visualized using a chemiluminescent substrate (ECL; Amersham, Arlington Heights, IL, United States) and the blots were exposed to Kodak X-OMAT film.

The signals in the Western blotting were quantified by densitometric scanning and normalized by using the intensity of corresponding protein band relative to the actin band.

Quantification of apoptosis
Quantitative detection of apoptotic cells was performed using two different methods: the nuclear binding dye DAPI and fluorescence microscopy, and the APO Per-
percentage apoptosis assay kit (Biocolor Ltd., Belfast, Northern Ireland). For the APO Percentage apoptosis assay, the cells were seeded at 10^4 cells per well in a 96-well plate and processed according to the manufacturer’s instructions.

Statistical analysis
All data were from at least three independent experiments using cells from a minimum of three separate isolations, and are expressed as the mean ± SD. Differences between the groups were compared using a two-tailed Student’s t tests or the Mann-Whitney U test as appropriate. P values of < 0.05 were considered to be statistically significant.

RESULTS
HMGB1 significantly increased cellular apoptosis in Huh-BAT cells in a dose- and time-dependent manner (Figure 1A and B). We repeated the same experiments in the other two hepatoma cell lines (HepG2 and SNU-475 cells) and observed the same effects (data not shown). We next identified the pro-apoptotic signaling pathways induced by HMGB1 treatment. HMGB1 increased cytochrome c release from mitochondria into cytosol and induced the cleavage of procaspase 3. However, it did not affect the activation of caspase 8, an initiator caspase downstream of death receptor activation (Figure 1C).

Since the MAPK family signaling cascades regulate apoptotic pathways, we next evaluated whether HMGB1 modulates MAPK activation. HMGB1 induced the activation of MAPKs such as p42/44, p38 MAPK, and JNK in Huh-BAT cells (Figure 2A). In order to explore the role of individual MAPKs in apoptotic signaling, the cells were then treated with HMGB1 either in the presence or absence of various inhibitors: U0126 for p42/44, SB203580 for p38, and SP600125 for JNK. When the cells were treated with the p38 inhibitor, HMGB1-induced caspase 3 activation was significantly attenuated whereas treatment with inhibitors of p42/44 or JNK did not affect caspase 3 cleavage (Figure 2B).

Pretreatment with GL significantly attenuated HMGB1-induced hepatocyte apoptosis in a dose-dependent manner (Figure 3A). GL also attenuated cytochrome c release from the mitochondria into cytosol (Figure 3B). Finally, pretreatment with GL decreased HMGB1-induced p38
activation in Huh-BAT cells (Figure 3C). Taken together, all of the findings from our study indicate that HMGB1 induces hepatocyte apoptosis through a p38-dependent mitochondrial pathway which was inhibited by GL.

**DISCUSSION**

In virtually all human liver diseases, hepatocytes undergo cell death by apoptosis [13]. Thus, therapeutic modulation of apoptosis has the potential to alter the course of human liver disease. Apoptosis may occur via two fundamental pathways: (1) the death receptor or extrinsic pathway, and (2) the mitochondrial or intrinsic pathway. Caspases, representing the family of cysteine proteases, play a critical role in both pathways. Both pathways can either directly or indirectly converge to activate the “effector caspase”, namely caspase-3, which induces DNA fragmentation and other morphological changes characteristic of apoptotic cell death [23]. In the present study, HMGB1 activated caspase 3 without affecting caspase 8, an initiator caspase downstream of death receptor activation. These findings suggest that the mitochondrial pathway is responsible for HMGB1-induced hepatocyte apoptosis, which was further supported by the findings that HMGB1 increased cytochrome c release from mitochondria into the cytosol.

MAPKs, which include p42/44, p38, and JNK, are involved in pro-apoptotic signal transduction as well as cell growth and differentiation [23]. It has been previously shown that the p38 MAPK cascade promotes either cell death or cell survival [23,24] depending on the cell type and the kinase isoforms activated by various stress stimuli [25]. There is abundant evidence that p38 participates in cellular apoptosis [26,27] with one mechanism being the modulation of Bcl-2 protein family members to maintain an apoptotic checkpoint for mitochondrial dysfunction and cytochrome c release [28,29]. Likewise, in the present study HMGB1-induced hepatocyte apoptosis occurred through a mitochondrial pathway which was p38-dependent.

GL, a triterpene glycoside extracted from licorice root (Glycyrrhiza glabra), consists of one molecule of 18b-glycyrrhetinic acid and two molecules of glucuronic acid having the structure 18-b-glycyrrhetinic acid-3-O-b-D-glucuronopyranosyl-(1/2)-b-D-glucuronide. It is known that this molecule has a variety of hepato-protective properties in terms of anti-inflammatory, antiviral, and anti-tumor effects [17]. In an animal model of acute liver injury induced by carbon tetrachloride, GL reduced the serum tumor necrosis factor-alpha (TNF-α) level and alleviates acute liver injury [18]. Moreover, GL has anti-inflammatory effects on lipopolysaccharide (LPS)-induced acute liver injury through inhibition of TNF-α release [30]. Recently, Ikeda et al. [31] reported that GL reduced the number of TUNEL-positive cells in cases of acute hepatitis induced by LPS/D-GalN, anti-apoptotic effects of GL were found to be caspase-independent, and probably achieved
A: Huh-BAT cells were pretreated with glycyrrhizin (GL) (0, 0.1, 0.5, 1.0 and 2.0 mmol/L) for 12 h. Cells were then treated with 10 μg/mL of high-mobility group box 1 (HMGB1) for 6 h. Apoptosis was quantified by 4',6-diamidino-2-phenylindole staining and fluorescence microscopy. Data are expressed as the mean ± SD of three individual experiments. *P < 0.05, vs GL 0 mmol/L; B: Huh-BAT cells were pretreated with GL (2.0 mmol/L) for 12 h prior to adding HMGB1 (0-10 μg/mL). Mitochondrial and cytosolic extracts were isolated after 6 h of HMGB1 treatment, and equivalent amounts of cytosolic protein were immunoblotted with an anti-cytochrome c antibody. Immunoblot analysis using an anti-actin antibody was performed as a control for protein loading; C: Huh-BAT cells were pretreated with GL (2.0 mmol/L) for 12 h prior to being incubated with HMGB1. Cells were then lysed at the indicated time points, and immunoblot analysis was performed using antibodies specific for the phosphorylated forms of p38 and actin.

Figure 3 Glycyrrhizin attenuates high-mobility group box 1-induced hepatocyte apoptosis. A: Huh-BAT cells were pretreated with glycyrrhizin (GL) (0, 0.1, 0.5, 1.0 and 2.0 mmol/L) for 12 h. Cells were then treated with 10 μg/mL of high-mobility group box 1 (HMGB1) for 6 h. Apoptosis was quantified by 4',6-diamidino-2-phenylindole staining and fluorescence microscopy. Data are expressed as the mean ± SD of three individual experiments. *P < 0.05, vs GL 0 mmol/L; B: Huh-BAT cells were pretreated with GL (2.0 mmol/L) for 12 h prior to adding HMGB1 (0-10 μg/mL). Mitochondrial and cytosolic extracts were isolated after 6 h of HMGB1 treatment, and equivalent amounts of cytosolic protein were immunoblotted with an anti-cytochrome c antibody. Immunoblot analysis using an anti-actin antibody was performed as a control for protein loading; C: Huh-BAT cells were pretreated with GL (2.0 mmol/L) for 12 h prior to being incubated with HMGB1. Cells were then lysed at the indicated time points, and immunoblot analysis was performed using antibodies specific for the phosphorylated forms of p38 and actin.

through the prevention of an interleukin-18-mediated inflammatory response. In the present study, we demonstrated that GL attenuated HMGB1-induced hepatocyte apoptosis by blocking the p38-dependent mitochondrial pathway. Therefore, it is likely that the hepatoprotective effects of GL are attributed to various mechanisms.

In summary, the present study demonstrated that HMGB1 participated in hepatocyte apoptosis through a p38-dependent mitochondrial pathway. In addition, GL had an anti-apoptotic effect on hepatocytes treated with HMGB1. Therefore, HMGB1 inhibitors, including GL, might be therapeutically efficacious in treating HMGB1-mediated liver injury such as viral hepatitis, hepatic ischemia-reperfusion injury and sepsis-related liver injury.

**Background**

High mobility group box 1 (HMGB1) is an evolutionarily conserved protein present in the nucleus of almost all eukaryotic cells, where it is involved in DNA replication, repair and transcription. Glycyrrhizin (GL) is a major active constituent of licorice root that is commonly used in Asia to treat patients with chronic hepatitis. This compound has been associated with numerous pharmacologic effects, including anti-inflammatory, anti-viral, anti-tumor, and hepatoprotective activities. Recently, GL was recognized as an HMGB1 inhibitor, which binds directly to both HMG boxes in HMGB1.

**Research frontiers**

The authors provide in vitro evidence and a potential theoretical basis for HMGB1 regulation of hepatocyte apoptosis in order to further elucidate the molecular mechanism of HMGB1 involvement in various pathologic conditions that can affect the liver. Furthermore, they attempted to determine whether GL attenuates HMGB1-induced hepatocyte apoptosis and, if so, to identify the signaling cascades responsible for this modulation.

**Innovations and breakthroughs**

Present study demonstrated that HMGB1 participated in hepatocyte apoptosis through a p38-dependent mitochondrial pathway. In addition, GL had an anti-apoptotic effect on hepatocytes treated with HMGB1.

**Applications**

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**Peer review**

This is an interesting study where the authors show that HMGB1 induces hepatocyte apoptosis which is mediated by p38. In addition, glycyrrhizin was shown to inhibit HMGB1-induced apoptosis as well as activation of p38 in the

**COMMENTS**

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High mobility group box 1 (HMGB1) is an evolutionarily conserved protein present in the nucleus of almost all eukaryotic cells, where it is involved in DNA replication, repair and transcription. Glycyrrhizin (GL) is a major active constituent of licorice root that is commonly used in Asia to treat patients with chronic hepatitis. This compound has been associated with numerous pharmacologic effects, including anti-inflammatory, anti-viral, anti-tumor, and hepatoprotective activities. Recently, GL was recognized as an HMGB1 inhibitor, which binds directly to both HMG boxes in HMGB1.

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cultured hepatocyte cell line. The study is well conducted and the manuscript is well written.

REFERENCES

1 Bianchi ME, Beltrame M. Flexing DNA: HMG-box proteins and their partners. Am J Hum Genet 1998; 63: 1573-1577

2 Bustin M, Reeves R. High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function. Prog Nucleic Acid Res Mol Biol 1996; 54: 35-100

3 Müller S, Scaffidi P, Degryse B, Bonaldi T, Ronfani L, Agresti A, Beltrame M, Bianchi ME. New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. EMBO J 2001; 20: 4337-4340

4 Wang H, Bloom O, Zhang M, Vishnubhatla JM, Ombrello M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ, HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285: 248-251

5 Wittemann B, Neuer G,Michel H, Truckenbrodt H, Bautz FA. Autoantibodies to nonhistone chromosomal proteins HMG-1 and HMG-2 in sera of patients with juvenile rheumatoid arthritis. Arthritis Rheum 1990; 33: 1378-1380

6 Kokkola R, Sundberg E, Uhligk AR, Palmblad K, Li J, Wang H, Ulloa L, Yang H, Yan Xj, Furie R, Chiorazzi N, Tracey KJ, Andersson U, Harris HE. High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. Arthritis Rheum 2002; 46: 2598-2603

7 Baldassarre G, Battista S, Belletti B, Thakur S, Pentimalli F, Trappasso F, Fedele M, Pierantoni G, Croce CM, Fusco A. Negative regulation of BRCA1 gene expression by HMG1 proteins accounts for the reduced BRCA1 protein levels in sporadic breast carcinoma. Mol Cell Biol 2003; 23: 2225-2238

8 Bussemakers MJ, van den Wj, Debruyne FM, Schalken JA. Identification of high mobility group protein I(Y) as potential progression marker for prostate cancer by differential hybridization analysis. Cancer Res 1991; 51: 606-611

9 Kim JY, Park JS, Strassheim D, Douglas I, Diaz-del Valle F, Asehnoune K, Mitra S, Kwak SH, Yamada S, Maruyama I, Ishizaka A, Abraham E. HMGB1 contributes to the development of acute lung injury after hemorrhage. Am J Physiol Lung Cell Mol Physiol 2005; 289: L1985-L1986

10 Levy RM, Mollen KP, Prince JM, Kaczorowski DJ, Vodovotz Y, Billiar TR. Systemic inflammation and remote organ injury following trauma require HMGB1. Am J Physiol Regul Integr Comp Physiol 2007; 293: R1538-R1544

11 Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller Da, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med 2005; 201: 1135-1143

12 Wang H, Ward MF, Fan XG, Sama AE, Li W. Potential role of high mobility group box 1 in viral infectious diseases. Viral Immunol 2006; 19: 3-9

13 Yoon JH, Gores GJ. Death receptor-mediated apoptosis and the liver. J Hepatol 2002; 37: 400-410

14 Yamamura Y, Klotki H, Tanaka N, Aikawa T, Sawada Y, Iga T. The pharmacokinetics of glycyrrhizin and its restorative effect on hepatic function in patients with chronic hepatitis and in chronically carbon-tetrachloride-intoxicated rats. Biopharm Drug Dispos 1997; 18: 717-725

15 Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, Saitoh S, Kobayashi M, Kumada H. The long term efficacy of glycyrrhizin in chronic hepatitis C patients. Cancer 1997; 79: 1494-1500

16 van Rossum TG, Vullo AG, Hop WC, Schalm SW. Glycyrrhizin-induced reduction of ALT in European patients with chronic hepatitis C. Am J Gastroenterol 2001; 96: 2432-2437

17 Sato H, Goto W, Yamamura J, Kurokawa M, Kageyama S, Takahara T, Watanabe A, Shiraki K. Therapeutic basis of glycyrrhizin on chronic hepatitis B. Antiviral Res 1996; 30: 171-177

18 Sittia G, Iannaccone M, Müller S, Bianchi ME, Guidotti LG. Treatment with HMGB1 inhibitors diminishes CTL-induced liver disease in HBV transgenic mice. J Leukoc Biol 2007; 81: 100-107

19 Higuchi H, Bronk SF, Takaykawa Y, Werneburg N, Takimoto R, El-Deiry W, Gores GJ. The bile acid glycocineodeoxycholeate induces trit-cceptor 2/DR5 expression and apoptosis. J Biol Chem 2001; 276: 38610-38618

20 Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J. Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Cancer Res 1982; 42: 3585-3586

21 Park JG, Lee JH, Kang MS, Park KJ, Jeon YM, Lee HJ, Kwon HS, Park HS, Yeo KS, Lee KU. Characterization of cell lines established from human hepatocellular carcinoma. Int J Cancer 1995; 62: 276-282

22 Jänicke RU, Sprengart ML, Wati MR, Porter AG. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 1998; 273: 9357-9360

23 Liu G, Zhang Y, Bode AM, Ma WY, Dong Z. Phosphorylation of 4E-BP1 is mediated by the p38/MSK1 pathway in response to UVB irradiation. J Biol Chem 2002; 277: 8201-8206

24 Sarkar D, Su ZZ, Lebedeva IV, Savae M, Gopalakrishnan RV, Valerie K, Dent F, Fisher PB. md-a (IL-24) Mediates selective apoptosis in human melanoma cells by inducing the coordinated overexpression of the GADD family of genes by means of p38 MAPK. Proc Natl Acad Sci USA 2002; 99: 10054-10059

25 Giafas N, Katsoulidis E, Sassano A, Tallman MS, Higgins LS, Nebreda AR, Davis RJ, Platianis LC. Role of the p38 mitogen-activated protein kinase pathway in the generation of arsenic trioxide-dependent cellular responses. Cancer Res 2006; 66: 6763-6771

26 Wada T, Pennington JM. Mitogen-activated protein kinases in apoptosis regulation. Oncogene 2004; 23: 2838-2849

27 Zarubin T, Han J. Activation and signaling of the p38 MAPK pathway. Cell Res 2005; 15: 11-18

28 Bennc SC, Woolf CJ. Adult neuron survival strategies--slamming on the brakes. Nat Rev Neurosci 2004; 5: 686-700

29 Ghatan S, Larner S, Kinoshita Y, Hetman M, Patel L, Xia Z, Youle RJ, Morrison RS. p38 MAPK mediates bax translocation in nitric oxide-induced apoptosis in neurons. J Cell Biol 2001; 150: 335-347

30 Lee CH, Park SW, Kim YS, Kang SS, Kim JA, Lee SH, Lee SM. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. Biol Pharm Bull 2007; 30: 1898-1904

31 Tang B, Qiao H, Meng F, Sun X. Glycyrrhizin attenuates endotoxin-induced acute liver injury after partial hepatectomy in rats. Braz J Med Biol Res 2007; 40: 1637-1646

32 Ikeda T, Abe K, Kuroda N, Kida Y, Inoue H, Wake K, Morito M, Sato T. The inhibition of apoptosis by glycyrrhizin in hepatic injury induced by injection of lipopolysaccharide / D-galactosamine in mice. Arch Histol Cytol 2008; 71: 163-178

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