ORIGINAL ARTICLE

Prevention of acetaminophen-induced hepatocyte injury: JNK inhibition and GSTA1 involvement

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Abstract

Background Glutathione S-transferase A1 (GSTA1) is a detoxification enzyme and a sensitive marker for hepatotoxicity. We investigated the effects of JNK inhibition on different degrees of Acetaminophen (APAP)-induced hepatocyte injury and GSTA1 expression.

Objective This study aimed to investigate the role of JNK signaling pathway in APAP-induced different degrees of hepatocyte injury and its correlation with GSTA1 by inhibiting the phosphorylation of JNK by SP600125.

Results 6 and 8 mM APAP induced different degrees of hepatocyte injury and apoptosis, both activated JNK signaling pathway. In contrast, JNK inhibitor significantly reduced activation of JNK and c-JUN on exposure to APAP. Meanwhile, the levels of hepatocyte injury, oxidative stress, and apoptosis obviously decreased. Importantly, GSTA1 expression was significantly increased by JNK inhibition.

Conclusions JNK inhibition attenuates APAP-induced hepatocyte injury and oxidative stress and increases GSTA1 expression. Furthermore, GSTA1 may be involved in this signaling pathway for detoxification.

Keywords JNK · GSTA1 · Acetaminophen · SP600125 · Hepatocyte injury

Introduction

Drug-induced liver injury is a liver function damage caused by exposure to drugs in the absence of other infectious factors and has become one of the most common clinical diseases (Ma et al. 2018). Acetaminophen (APAP) is an antipyretic and analgesic that can relieve mild to moderate pain from colds, as well as severe pain such as cancer pain and postoperative pain (Giamarellos-Bourboulis et al. 2014). The APAP-induced liver injury model is one of the

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common models for studying drug-induced liver injury (Roh et al. 2018).

The c-Jun N-terminal kinase (JNK) signaling pathway is involved in mitogen-activated protein kinase (MAPK) (Lawan and Bennett 2017). Apoptosis signal-regulating kinase 1 (ASK1), a MAP kinase kinase kinase, is located upstream of JNK and capable of phosphorylating MKK4/7 (MAP kinase kinases). MKK4/7 in turn activates JNK by dual phosphorylation of Thr[183]/Tyr[185] of JNK (Liu et al. 2000). Moreover, JNK is thought to bind and phosphorylate the Ser-63 and Ser-73 residues of c-JUN within its transcriptional activation domain (Sergi et al. 2019). c-JUN can self-assemble to form homodimers or form heterodimers with other transcription factors, such as c-FOS (Nadeem et al. 2018). These dimers are also known as activator protein 1 (AP-1). JNK is involved in the regulation of critical cellular functions, such as growth, differentiation, survival and apoptosis, by activating and inhibiting downstream transcription factors (Huang et al. 2015).

Eight glutathione S-transferases (GSTs) families have been identified in the human body: Alpha, Delta, Kappa, Mu, Omega, Pi, Theta, Zeta (Chang et al. 2017). GSTA1, a member of GST Alpha family, highly expressed in normal liver (about 65–75% of GSTs) and kidney tissue (Mücher et al. 1998). GSTA1 plays a key role in the protection of oxidative stress damage and catalyzes the binding of GSH to carcinogens, drugs, and oxidative stress toxins (Adnan et al. 2012). Furthermore, there might be a potential relationship between JNK signaling pathway and GSTA1.

SP600125 (1,9-pyrazolone), an inhibitor of JNKs, is used in biochemical studies and is a reversible membrane JNK inhibitor with high selectivity and potency (Bennett et al. 2001). Therefore, this study investigated the role of JNK signaling pathway in APAP-induced different degrees of hepatocyte injury and its correlation with GSTA1 by inhibiting the phosphorylation of JNK by SP600125.

Materials and methods

Cell culture and viability

HepG2 cells were obtained from Harbin Medical University Pharmacology Laboratory and maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. All cells were cultured at 37 °C in a humidified 5% CO_2 cell culture incubator.

Replication of hepatocyte injury model induced by APAP

Hepatocytes were plated in 24-well plates at a density of 1.5×10^5 cells/well and cultured for 12 h. Then, hepatocytes

were treated with DMEM solution contained 0, 4, 6, 8, 10, and 12 mM APAP (Aladdin Bio-Chem Technology Co., Ltd, Shanghai, China) for 6 h. Each group contained four repetitions. Finally, the supernatant was collected for detection of transaminases (ALT, AST) activities.

Optimum concentration screening for SP600125

Hepatocytes were plated in 24-well plates at a density of 1.5×10^5 cells/well and cultured for 12 h. Hepatocytes were divided into six groups, including control group, 8 mM APAP group, SP600125 (SP) (1 µM, 2 µM and 5 µM) + APAP groups and SP (5 µM) group. SP (Absin Bioscience Inc, Shanghai, China) was dissolved in DMSO to 50 mM, and diluted in medium to obtain the required concentrations (1, 2 and 5 µM). The control group was given an equal volume of DMSO. The final concentration of DMSO was not more than 0.01%. The cells were taken after 6 h of exposure to APAP and SP to determine the transaminases (ALT, AST) activity.

Effects of SP600125 on APAP-induced hepatocyte injury

Hepatocytes were plated in 6-well plates at a density of 6×10^5 cells/well and cultured for 12 h. Hepatocytes were divided into five groups that were control group, 6 mM APAP group, 8 mM APAP group, SP (2 µM) + 6 mM APAP group, SP (2 µM) + 8 mM APAP group. The supernatant and cells were taken after 6 h of exposure to APAP and SP.

ALT, AST, MDA, SOD, GSH and GSH-Px levels detection

As previously described (Chang et al. 2019), ALT, AST, MDA, SOD, GSH and GSH-Px levels were measured using corresponding assay kit (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China) according to the manufacturer's instructions.

Hoechst 33342 staining

Hoechst 33342 is a DNA-specific fluorescent dye that penetrates the cell membrane and binds it through the A-T bond of DNA. Hoechst 33342 (Beyotime Biotechnology Co., Ltd, Shanghai, China) staining was used to investigate the changes in nuclear morphology of apoptosis, observed by fluorescence microscopy. After treatment for 6 h, the culture supernatant was abandoned. Hepatocytes were stained with 1 ml Hoechst 33342 to each well at 37 °C for 30 min, washed with PBS and observed under a fluorescence microscope.

Western blot analysis

The cells were lysed on the surface of ice in cell lysis buffer supplemented with 1 mM PMSF. Cells were then homogenized using a 1 ml syringe. Cell lysates were centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was collected, and total protein concentration was determined using a BCA Protein Assay Kit (Beyotime Biotechnology Co., Ltd, Shanghai, China). The protein of each sample was adjusted to be the same concentration and conventional western blot processes were performed as previously described (Ma et al. 2018). Rabbit anti-GSTA1, anti-JNK1/2/3, anti-p-JNK1/2/3 (Thr183 + Tyr185), anti-c-JUN, anti-p–c-JUN (Ser243), anti-MKK4, anti-p-MKK4, anti-ASK1, anti-p-ASK1, anti-c-FOS, anti-p–c-FOS antibody (Affinity Biosciences Co., Ltd, USA) and Mouse anti-β-actin antibody (Zhongshanjinqiao Biotechnology Co., Ltd, Beijing, China) were used in this study. Total gray values of each band were digitized using Image J software. The relative expression level of each protein was normalized using β-actin as a reference gene.

Statistical analysis

Analysis of data expressed as the mean ± SD and was performed using one-way analysis of variance using the SPSS software 19.0. The Duncan multiple comparison test was used to examine the statistical significance ($p < 0.05$ and $p < 0.01$) between groups.

Results

Replication of APAP-induced hepatocyte injury model

To replicate APAP-induced hepatocyte injury model, after hepatocytes were exposed to different concentrations of APAP, ALT and AST activities in the supernatant were measured (Fig. 1a, b). Compared to control group, significant increase has been noted at both 6 mM ($p < 0.05$) and 8 mM ($p < 0.01$) APAP. Therefore, 6 mM and 8 mM APAP were used in subsequent studies to induce mild hepatocyte injury and severe hepatocyte injury, respectively.

Optimal concentration screening for JNK inhibitor SP600125

To determine the optimal concentration of JNK inhibitor, ALT and AST activities were measured in supernatant exposed to 8 mM APAP with 1, 2 and 5 μM SP
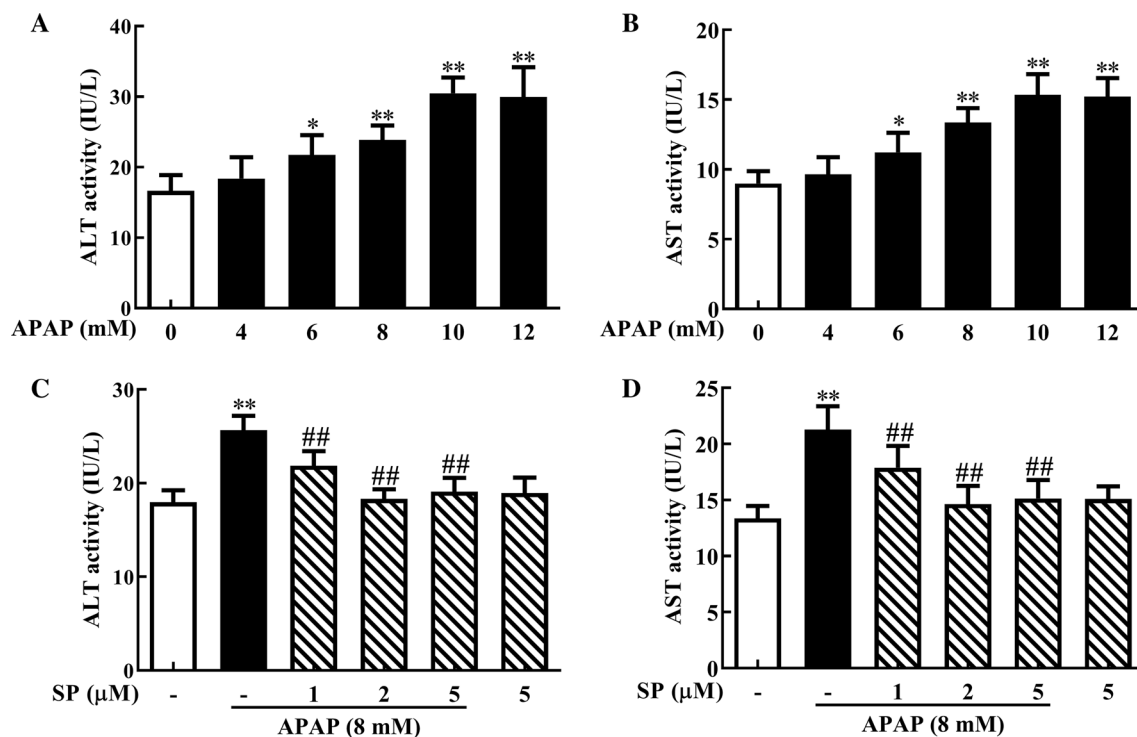

Fig. 1 Optimal concentration screening for APAP and JNK inhibitor SP. Changes of ALT activity (**a**) and AST activity (**b**) in culture supernatant after APAP exposure (after 6 h) at various concentrations (0 mM, 4 mM, 6 mM, 8 mM, 10 mM, 12 mM, $n = 4$). Changes of ALT activity (**c**) and AST activity (**d**) in culture supernatant in exposure to 8 mM APAP with 1, 2 and 5 μM SP600125 at 6 h ($n = 4$). *$p < 0.05$ and **$p < 0.01$ (compared to control group). #$p < 0.05$ and ##$p < 0.01$ (compared to APAP group). The same below

co-treatment (shown in Fig. 1c, d). Compared to control group, ALT and AST activities significantly increased ($p < 0.01$) with exposure to APAP (8 mM). Compared to APAP group, ALT and AST activities significantly decreased ($p < 0.01$) in 1, 2 and 5 μM SP + APAP groups. Among them, levels of transaminase activities reached minimum in 2 μM SP + APAP group. Therefore, 2 μM SP600125 was used in subsequent studies to intervene different degree of APAP-induced hepatocyte injury.

SP600125 inhibited upstream protein activation of JNK signaling pathway

To explore whether SP inhibited JNK signaling pathway in the concentration of 2 μM, the activation of JNK and its upstream and downstream proteins expression were measured (Fig. 2). As shown in Fig. 2b, c, 6 mM and 8 mM APAP significantly induced ($p < 0.01$) the activation of ASK1 (ratio of p-ASK1/ASK1) and MKK4 (ratio of p-MKK4/MKK4) compared to control group. While, interestingly, 2 μM SP did not inhibit the activation levels of ASK1 and MKK4, except for hepatocytes in 8 mM APAP + SP group compared to APAP group.

SP600125 inhibited activation of JNK and its downstream proteins

The results of SP inhibited activation of JNK and its downstream proteins have been shown in Fig. 2d−f. Compared to control group, 6 mM and 8 mM APAP markedly induced ($p < 0.01$) activation of JNK (ratio of p-JNK/JNK), c-JUN (p–c-JUN/c-JUN) and c-FOS (p–c-FOS/FOS). Conversely, compared to APAP groups, a significant decrease was observed in the activation of JNK ($p < 0.01$) and c-JUN ($p < 0.05$) in 6 mM APAP + SP group. Moreover, an obvious decrease was observed in the activation of JNK ($p < 0.05$) and c-JUN ($p < 0.01$) in 8 mM APAP + SP group. Interestingly, there was no significant change in the activation of c-FOS in 6 and 8 mM APAP + SP groups.

JNK inhibitor mitigated different degree APAP-induced hepatocyte injury

Subsequently, we measured ALT, AST, MDA, GSH, GSH-Px and SOD levels to determine whether inhibition of JNK mitigated different degree APAP-induced hepatocyte injury. Compared to control group, ALT and AST activities in supernatant and MDA content in hepatocytes were significantly increased ($p < 0.05$). GSH content, GSH-Px and
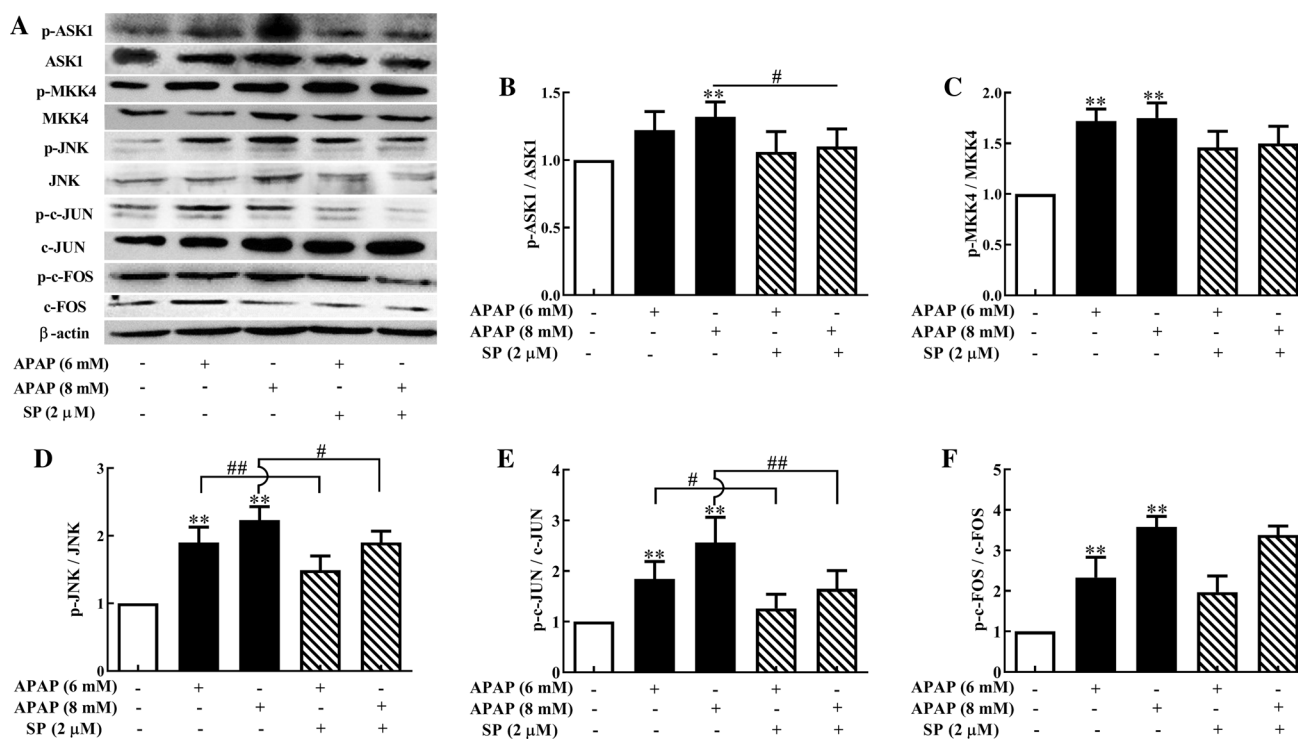

Fig. 2 Effects of SP on the activation of JNK signaling pathway. Original blots for p-ASK1, ASK1, p-MKK4, MKK4, p-JNK, JNK, p–c-JUN, c-JUN, p–c-FOS and c-FOS (**a**). The activation of ASK1 (ratio of p-ASK1/ASK1, **b**), MKK4 (ratio of p-MKK4 and MKK4, **c**), JNK (ratio of p-JNK/JNK, **d**), c-JUN (ratio of p–c-JUN/c-JUN, **e**) and c-FOS (ratio of p–c-FOS/c-FOS, **f**) in hepatocytes exposed to 6 mM and 8 mM APAP with 2 μM SP

SOD activities in hepatocytes were obviously decreased ($p < 0.05$) on exposure to 6 mM APAP and these indicators were significantly changed ($p < 0.01$) on exposure to 8 mM APAP. Compared to APAP groups, ALT and AST activities in supernatant and MDA content in hepatocytes significantly reduced ($p < 0.05$), GSH content, GSH-Px and SOD activities obviously enhanced ($p < 0.05$) in 6 mM APAP + SP group and these indicators were significantly changed ($p < 0.01$) in 8 mM APAP + SP group.

Effects of JNK inhibitor on hepatocyte apoptosis

To determine whether inhibition of JNK could resist APAP-induced apoptosis, Hoechst 33342 staining was used to observe the changes in hepatocyte apoptosis after administration of JNK inhibitor SP600125 in APAP-induced hepatocyte injury by fluorescence microscopy (Fig. 3g). The nucleus of normal cells was uniformly lightly stained, while the nucleus of apoptotic cells was densely stained with
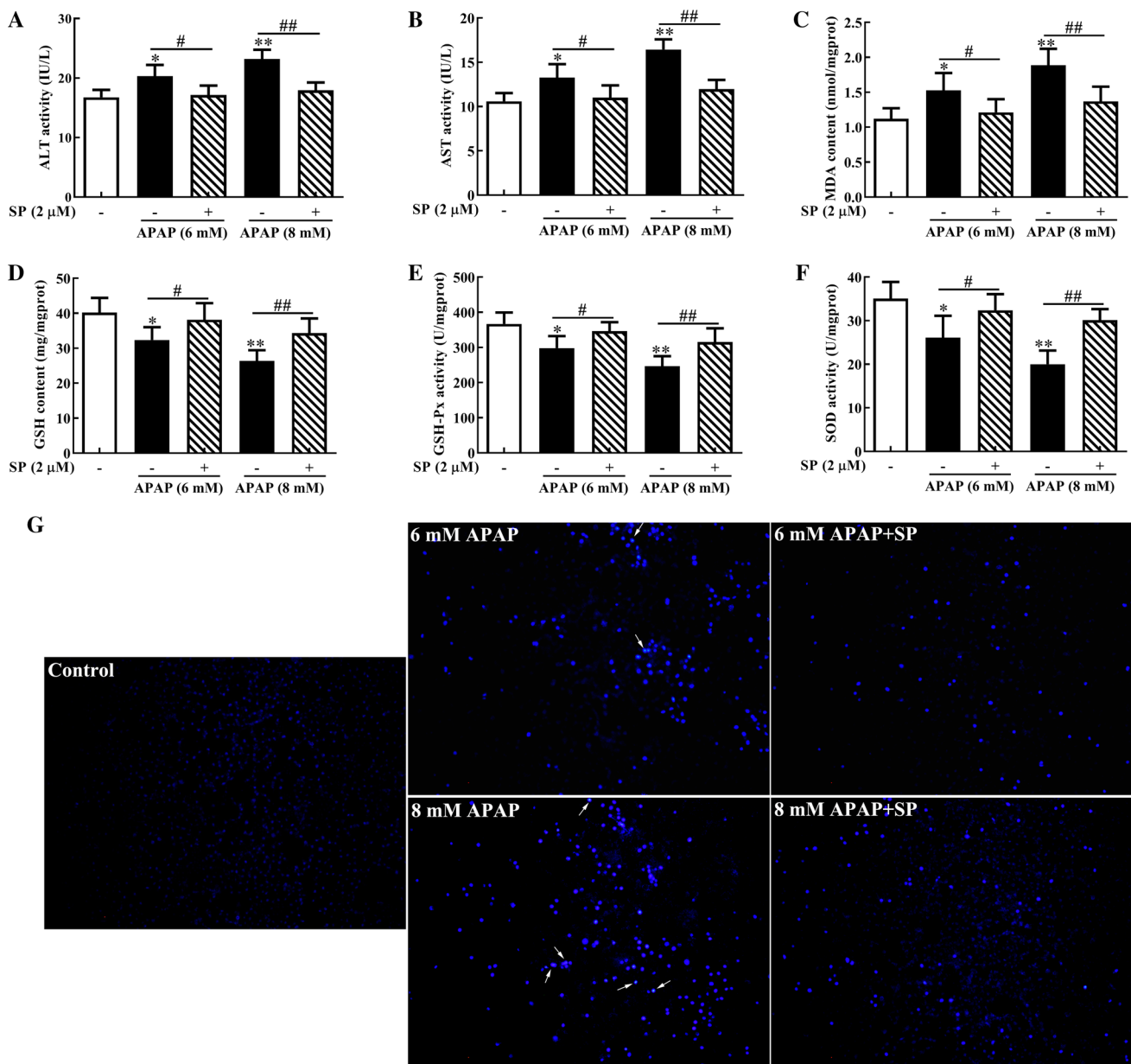

Fig. 3 Intervention of SP on APAP-induced hepatocyte injury, oxidative stress and apoptosis. Changes of ALT (**a**) and AST (**b**) activities in culture supernatant, MDA (**c**) and GSH (**d**) contents, GSH-Px (**e**) and SOD (**f**) activities in hepatocytes exposed to 6 mM and 8 mM APAP with 2 μM SP ($n = 4$). Hoechst 33342 was used to nuclear staining of apoptotic cells in hepatocytes. The stained cells were examined using fluorescence microscopy (**g**) in control group, 6 mM APAP group, 8 mM APAP group, 6 mM APAP + 2 μM SP group and 8 mM APAP + 2 μM SP group

fragments or non-fragments. The nuclei in control hepatocytes appeared normal and exhibited mild diffuse staining of the chromatin. While, as shown by the arrow, 6 mM and 8 mM APAP obviously induced apoptosis which showed bright and dense fluorescence. In addition, the nucleus in 6 mM or 8 mM APAP+SP groups tended to be normal with only a few densely stained nuclei, indicating that the apoptosis was significantly reduced by co-treatment of 2 μM SP in APAP-induced hepatocyte injury.

Effects of JNK inhibitor on GSTA1 expression in APAP-induced hepatocyte injury

Finally, GSTA1 expression has been measured in cells exposed to 6 and 8 mM APAP with 2 μM SP co-treatment to explore whether inhibition of JNK could regulate GSTA1 expression (Fig. 4). Compared to control, GSTA1 protein expression significantly decreased with 6 mM and 8 mM APAP-treatment. Compared to APAP groups, importantly, GSTA1 expression was obviously enhanced in 6 and 8 mM APAP+SP groups.

Discussion

Our previous results showed that GSTA1 appeared earlier than ALT and AST in liver injury and could be used as an early marker of liver injury (Liu et al. 2014; Chang et al. 2017). Meanwhile, GSTA1 is also a more sensitive marker in APAP-induced hepatocyte injury (Li et al. 2017). HepG2 cells are commonly used to study APAP-induced hepatotoxicity (Roh et al. 2018). Therefore, this study aims to investigate the role of JNK signaling pathway in different degree of APAP-induced hepatocyte injury and its correlation with GSTA1 expression.

Acetaminophen is rapidly absorbed after oral administration and mainly metabolized in the liver. A part of APAP is metabolized to N-acetyl-p-benzoquinone imine (NAPQI), a metabolite that is generally considered to be toxic by APAP (Shi et al. 2017). In addition, ROS produced during APAP metabolism induce subsequent lipid peroxidation and liver damage. In this study, ALT and AST activities in the culture supernatant were increased in exposure to APAP in a dose-dependent manner. 6 mM and 8 mM APAP induced different degrees of elevation of AST and ALT, indicating that APAP-induced different degrees of hepatocyte injury models were successfully replicated and could be used in subsequent test.

ASK1 activates downstream MAPK kinase kinases (MKK) in response to a range of stresses such as oxidative stress, endoplasmic reticulum stress and calcium influx (Shiizaki et al. 2013). MKK4, a direct activator of MAPK, has been shown to activate JNK1, JNK2 and p38. Moreover, phosphorylated JNK transferred to the nucleus can phosphorylate nuclear transcription factors such as c-JUN, ATF-2, ELK-1 and p53, which induce the formation of the transcriptional complex AP-1 and regulate transcription of downstream apoptosis-related genes and expression of related proteins (Anilkumar and Prehn 2014). The activation of JNK cascade signaling has been found in APAP-induced hepatotoxicity (Ramachandran and Jaeschke 2019). We found that ALT and AST activity decreased to the minimum after 2 μM SP treatment. Therefore, 2 μM SP was selected for subsequent experiments. ALT and AST are traditional indicators for detecting liver injury, and are released into the extracellular space after cell membrane permeability changes (Liu et al. 2016). SOD, GSH and GSH-Px belong to enzymatic or non-enzymatic antioxidant systems and can resist oxidative stress (Sinthorn et al. 2016; Aldini et al. 2018). The content of MDA can be used to estimate the degree of lipid peroxidation in the body (Davey et al. 2005). After exposure of 8 mM
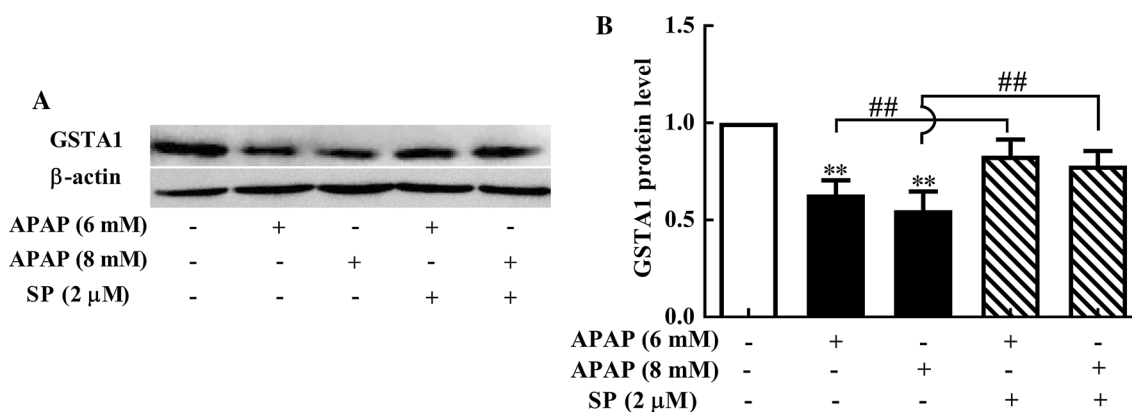

Fig. 4 Effects of SP on GSTA1 protein expression in APAP-induced hepatocyte injury. Original blots for GSTA1 and β-actin (**a**). GSTA1 protein level in hepatocytes exposed to 6 mM and 8 mM APAP with 2 μM SP (**b**)

APAP, ALT and AST were released into the supernatant in large amounts from the cells, a large amount of MDA was produced, and SOD, GSH, and GSH-Px were severely consumed. In contrast, SP can restore the storage of SOD, GSH, GSH-Px, reduce the MDA content, and protect APAP-induced hepatocyte injury, indicating that the antioxidant capacity of SP-treated hepatocytes was improved.

In addition, 2 μM SP treatment reduced the phosphorylation of JNK and c-JUN induced by 6 mM and 8 mM APAP, inhibited the transcriptional activity of c-JUN, and provided protection against different levels of hepatocyte injury induced by APAP. While, 2 μM SP did not inhibit phosphorylation of the JNK upstream proteins, ASK1 and MKK4, indicating that it had no obviously inhibitory effect on JNK upstream protein. Generally, c-JUN can be oligomerized to form a homodimer or polymerized with c-FOS to form a heterodimer which are called AP-1 (Thiel and Rössler 2014). Interestingly, activation of c-FOS was also not inhibited, suggesting that transcription of c-FOS may be regulated by other pathways, such as ERK (You et al. 2016). Apoptosis is a process of active, physiological death that exists in multicellular organisms and is a highly controlled process throughout the entire life cycle. After activation of apoptosis, various changes occur in cell state, such as cell shrinkage, nuclear fragmentation, chromatin condensation, and chromatin DNA fragmentation (Pamenter et al. 2013). Hoechst 33,342 staining was used to detect apoptosis in this study. After APAP metabolism, JNK was activated by oxidative stress and induces apoptosis. In contrast, SP treatment significantly inhibited apoptosis in hepatocytes.

In addition to metabolizing bilirubin and certain anticancer drugs in the liver, GSTA1 also has glutathione peroxidase activity, thereby protecting cells from ROS and peroxidation products (Sharma et al. 2011; Ma et al. 2017; Li et al. 2018). Both 6 mM and 8 mM APAP significantly decreased the expression of GSTA1 in hepatocytes. While, after the JNK signaling pathway was inhibited, the expression of GSTA1 increased suggesting that the activation of JNK has a negative regulatory effect on the expression of GSTA1 in APAP-induced hepatocyte injury. Furthermore, the specific mechanism by which the JNK signaling pathway is involved in the regulation of GSTA1 remains to be further studied.

In summary, JNK signaling pathway can be activated in different degrees of hepatocyte injury induced by 6 mM and 8 mM APAP. JNK inhibitor, SP600125, can inhibit the activation of JNK and its downstream protein but not upstream protein. Inhibition of JNK signaling pathway can attenuate APAP-induced hepatocyte injury and apoptosis, increase the expression of GSTA1 in hepatocytes, and improve the ability of hepatocytes to resist oxidative stress induced by APAP. Above all, inhibition of JNK signaling pathway has protective effects on APAP-induced hepatocyte injury, and GSTA1, which showed negative correlation with JNK, that could be involved in the detoxification mechanism of this signaling pathway.

Author contributions FL supervised the whole experiments. JH and YC performed the practical work and completed the experiments. YC and MI wrote the whole manuscript. BM, JW, RZ, LY JL, and CL provided help during the experiments.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights Not applicable.

References

Adnan H et al (2012) Low levels of GSTA1 expression are required for Caco-2 cell proliferation. PLoS ONE 7(12):e51739. https://doi.org/10.1371/journal.pone.0051739

Aldini G et al (2018) N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res 52(7):751–762. https://doi.org/10.1080/10715762.2018.1468564

Anilkumar U, Prehn JH (2014) Anti-apoptotic BCL-2 family proteins in acute neural injury. Front Cell Neurosci 8:281. https://doi.org/10.3389/fncel.2014.00281

Bennett BL et al (2001) SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. Proc Natl Acad Sci U S A 98(24):13681–13686. https://doi.org/10.1073/pnas.251194298

Chang Y et al (2019) Acetaminophen-induced hepatocyte injury: C2-ceramide and oltipraz intervention, hepatocyte nuclear factor 1 and glutathione S-transferase A1 changes. J Appl Toxicol 39(12):1640–1650. https://doi.org/10.1002/jat.3881

Chang YC et al (2017) Glutathione S-transferase A1 - a sensitive marker of alcoholic injury on primary hepatocytes. Hum Exp Toxicol 36(4):386–394. https://doi.org/10.1177/0960327116650013

Davey MW et al (2005) High-throughput determination of malondialdehyde in plant tissues. Anal Biochem 347(2):201–207. https://doi.org/10.1016/j.ab.2005.09.041

Giamarellos-Bourboulis EJ et al (2014) Intravenous paracetamol as an antipyretic and analgesic medication: the significance of drug metabolism. J Pharmacol Sci 124(2):144–152. https://doi.org/10.1254/jphs.13133FP

Huang W et al (2015) Paracrine Factors Secreted by MSCs Promote Astrocyte Survival Associated With GFAP Downregulation After Ischemic Stroke via p38 MAPK and JNK. J Cell Physiol 230(10):2461–2475. https://doi.org/10.1002/jcp.24981

Lawan A, Bennett AM (2017) Mitogen-Activated Protein Kinase Regulation in Hepatic Metabolism. Trends Endocrinol Metab 28(12):868–878. https://doi.org/10.1016/j.tem.2017.10.007

Li R et al (2017) Glutathione S-transferase A1 (GSTA1) as a marker of acetaminophen-induced hepatocyte injury in vitro. Toxicol Mech Methods 27(6):401–407. https://doi.org/10.1080/15376516.2017.1320457

Li Y et al (2018) Evaluation of hepatoprotective activity of Syringa oblata leaves ethanol extract with the indicator of glutathione S-transferase A1. Revista Brasileira de Farmacognosia. https://doi.org/10.1016/j.bjp.2018.05.011

Liu F et al (2014) Glutathione S-transferase A1 (GSTA1) release, an early indicator of acute hepatic injury in mice. Food Chem Toxicol 71:225–230. https://doi.org/10.1016/j.fct.2014.06.011

Liu FP et al (2016) Hepatoprotective effects of Solanum nigrum against ethanol-induced injury in primary hepatocytes and mice with analysis of glutathione S-transferase A1. J Chin Med Assoc 79:65–71. https://doi.org/10.1016/j.jcma.2015.08.013

Liu H, Nishitoh H, Ichijo H, Kyriakis JM (2000) Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. Mol Cell Biol 20(6):2198–2208. https://doi.org/10.1128/mcb.20.6.2198-2208.2000

Mücher G et al (1998) Fine Mapping of the Autosomal Recessive Polycystic Kidney Disease Locus (PKHD1) and the Genes MUT, RDS, CSNK2β, and GSTA1 at 6p21–p12. Genomics 48(1):40–45. https://doi.org/10.1006/geno.1997.5145

Ma X et al (2018) Effects of C2-Ceramide and Oltipraz on Hepatocyte Nuclear Factor-1 and Glutathione S-Transferase A1 in Acetaminophen-Mediated Acute Mice Liver Injury. Front Pharmacol 9:1009. https://doi.org/10.3389/fphar.2018.01009

Ma X et al (2017) Expression of glutathione S-transferase A1, a phase II drug-metabolizing enzyme in acute hepatic injury on mice. Exp Ther Med 14(4):3798–3804. https://doi.org/10.3892/etm.2017.4957

Nadeem L et al (2018) Differential expression of myometrial AP-1 proteins during gestation and labour. J Cell Mol Med 22(1):452–471. https://doi.org/10.1111/jcmm.13335

Pamenter ME et al (2013) DIDS (4,4-diisothiocyanatostilbenedisulphonic acid) induces apoptotic cell death in a hippocampal neuronal cell line and is not neuroprotective against ischemic stress. PLoS ONE 8(4):e60804. https://doi.org/10.1371/journal.pone.0060804

Ramachandran A, Jaeschke H (2019) Acetaminophen Hepatotoxicity. Semin Liver Dis 39(2):221–234. https://doi.org/10.1055/s-0039-1679919

Roh T et al (2018) Detoxifying effect of pyridoxine on acetaminophen-induced hepatotoxicity via suppressing oxidative stress injury. Food Chem Toxicol 114:11–22. https://doi.org/10.1016/j.fct.2018.02.017

Sergi C, Shen F, Liu SM (2019) Insulin/IGF-1R, SIRT1, and FOXOs Pathways-An Intriguing Interaction Platform for Bone and Osteosarcoma. Front Endocrinol (Lausanne) 10:93. https://doi.org/10.3389/fendo.2019.00093

Sharma R, Ellis B, Sharma A (2011) Role of alpha class glutathione transferases (GSTs) in chemoprevention: GSTA1 and A4 overexpressing human leukemia (HL60) cells resist sulforaphane and curcumin induced toxicity. Phytother Res 25(4):563–568. https://doi.org/10.1002/ptr.3297

Shi CX et al (2017) Hepatoprotective effects of ethanol extracts from Folium Syringae against acetaminophen-induced hepatotoxicity in vitro and in vivo. J Chin Med Assoc 80(10):623–629. https://doi.org/10.1016/j.jcma.2017.03.007

Shiizaki S, Naguro I, Ichijo H (2013) Activation mechanisms of ASK1 in response to various stresses and its significance in intracellular signaling. Adv Biol Regul 53(1):135–144. https://doi.org/10.1016/j.jbior.2012.09.006

Sinthorn W, Chatuphonprasert W, Chulasiri M, Jarukamjorn K (2016) Thai red rice extract provides liver protection in paracetamol-treated mice by restoring the glutathione system. Pharm Biol 54(5):770–779. https://doi.org/10.3109/13880209.2015.1079725

Thiel G, Rössler OG (2014) Resveratrol stimulates AP-1-regulated gene transcription. Mol Nutr Food Res 58(7):1402–1413. https://doi.org/10.1002/mnfr.201300913

You L, Ren X, Du Y, Zhao W, Cui M, Chen G, Zhao Y (2016) c-Fos/ERK promotes the progression from pancreatic intraepithelial neoplasia to pancreatic ductal adenocarcinoma. Oncol rep 36(6):3413–3420. https://doi.org/10.3892/or.2016.5169