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Citation for published version:

Digital Object Identifier (DOI):
10.1136/gut.2006.104372

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Gut

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Critical role of c-jun (NH2) terminal kinase in paracetamol-induced acute liver failure


Gut 2007;56:982–990. doi: 10.1136/gut.2006.104372

Paracetamol (acetaminophen) poisoning is the most common cause of acute liver failure (ALF) in the UK and US. Deaths from paracetamol-induced ALF still occur despite the availability of an effective antidote (N-acetylcysteine (NAC) or methionine) and changes in the sale and packaging of paracetamol in the UK. The only effective treatment for severe cases of paracetamol hepatotoxicity is orthotopic liver transplantation. However, transplantation has disadvantages including limited donors, the commitment of often young recipients to lifelong immunosuppression and psychiatric or medical contraindications in approximately 50% of patients. Effective alternative treatments for paracetamol-induced ALF are urgently required.

The metabolic activation of paracetamol to N-acetyl-p-quinoneimine (NAPQI) and subsequent conjugation with glutathione is well characterised. However, the pathways linking paracetamol metabolism with hepatic necrosis remain elusive. Recent investigations have expanded our understanding of the complex interplay between the pathways of toxin metabolism, intracellular signalling and the host inflammatory response. Inflammatory cells play a permissive role in hepatic necrosis induced by paracetamol and early-response cytokines and chemokines modulate both the injury and repair processes. The mitogen-activated protein kinases are a family of serine/threonine kinases that are evolutionarily conserved signal-transducing enzymes unique to eukaryotes. The c-jun (NH2) terminal kinases (JNKs) are one subgroup of the mitogen-activated protein kinases. The JNK signal transduction pathway is stimulated by a diverse range of extracellular and intracellular stimuli including reactive oxygen species (ROS), ultraviolet radiation and pro-inflammatory cytokines. JNK controls many basic mammalian physiological processes and phosphorylates specific subunits (c-Jun, JunB, JunD and activating transcription factor 2) of the activating protein 1 (AP-1) transcription factor, activating genes that regulate diverse cellular functions including proliferation, survival and death. Recent in vitro data and in vivo studies suggest a role for JNK in mediating hepatic injury. Using a previously developed mouse model of paracetamol poisoning, we demonstrate a central role for JNK in the pathogenesis of paracetamol-induced ALF, thereby identifying JNK as an important therapeutic target in the treatment of paracetamol hepatotoxicity.

Background: Acute hepatic failure secondary to paracetamol poisoning is associated with high mortality. C-jun (NH2) terminal kinase (JNK) is a member of the mitogen-activated protein kinase family and is a key intracellular signalling molecule involved in controlling the fate of cells.

Methods: A previously developed mouse model of paracetamol poisoning was used to examine the role of JNK in paracetamol-induced ALF.

Results: Paracetamol-induced hepatic JNK activation both in human and murine paracetamol hepatotoxicity and in our murine model preceded the onset of hepatocyte death. JNK inhibition in vivo (using two JNK inhibitors with different mechanisms of action) markedly reduced mortality in murine paracetamol hepatotoxicity, with a significant reduction in hepatic necrosis and apoptosis. In addition, delayed administration of the JNK inhibitor was more effective than N-acetylcysteine after paracetamol poisoning in mice. JNK inhibition was not protective in acute carbon tetrachloride-mediated or anti-Fas antibody-mediated hepatic injury, suggesting specificity for the role of JNK in paracetamol hepatotoxicity. Furthermore, disruption of the JNK1 or JNK2 genes did not protect against paracetamol-induced hepatic damage. Pharmacological JNK inhibition had no effect on paracetamol metabolism, but markedly inhibited hepatic tumour necrosis factor α (TNF α) production after paracetamol poisoning.

Conclusions: These data demonstrated a central role for JNK in the pathogenesis of paracetamol-induced liver failure, thereby identifying JNK as an important therapeutic target in the treatment of paracetamol hepatotoxicity.

MATERIALS AND METHODS

Human biopsy specimens and animal models

Archival human liver samples were obtained from the Department of Pathology, University of Edinburgh, Edinburgh, UK. All animal procedures were undertaken using C57/BL6 mice, with approved licence from the Animal Scientific Procedures Division of the Home Office (London, UK) using mice aged 8–10 weeks. Generation of JNK1−/− and JNK2−/− mice by gene-targeting technology (on a C57/BL6 background) has been described previously. As control, age- and sex-matched wild-type (WT) littermates were used. After an overnight fast, mice were injected intraperitoneally (IP) with 350 mg/kg of paracetamol dissolved in sterile phosphate-buffered saline (PBS). 4TJ, UK; k.simpson@ed.ac.uk

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Revised 22 November 2006
Accepted 6 December 2006
Published Online First
21 December 2006

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Abbreviations: ALF, acute liver failure; ALT, alanine aminotransferase; AP-1, activating protein 1; CCl4, carbon tetrachloride; c-jun, c-jun N-terminal kinase peptide inhibitor 1, c-stereoisomer; IP, intraperitoneal; JNK, c-jun (NH2) terminal kinase; NAC, N-acetylcysteine; NAPQI, N-acetyl-p-quinoneimine; PBS, phosphate-buffered saline; ROS, reactive oxygen species; TNFα, tumour necrosis factor α; TNFRI, TNF receptor 1; WT, wild type

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buffered saline (PBS) and warmed to 42°C as described previously,
with carbon tetrachloride (CCl4) at a ratio of 1:3
with olive oil at a dose of 1 μl/g body weight as described previously
or with anti-Fas antibody (0.5 μg/g body weight; BD Pharmingen, UK). Two JNK inhibitors were used in this study: SP600125 (anthr[1,9-cd]pyrazol-6(2H)-one) purchased from Calbiochem (San Diego, California, USA) and D-JNKI1 (c-Jun N-terminal kinase peptide inhibitor 1, β-steroisomer) inhibitor purchased from Axcora (Nottingham, UK). The vehicle control for SP600125 was 40% polyethylene glycol (PEG; Sigma, UK) in PBS. The control for D-JNKI1 was D-TAT control peptide (Axxora) in PBS. In experiments utilising delayed JNK inhibition, pharmacological inhibitors or appropriate controls were administered IP 5 h after paracetamol injection.

Analysis of liver injury
After IP injection, groups of mice were scored as follows by a blinded observer for signs of systemic illness: clinically well (0 points), presence of piloerection (1 point), with additional hunched posture (2 points) and lack of spontaneous movement (3 points) 24 h after injection. Serum was stored at −80°C until use for determination of alanine aminotransferase (ALT) levels by an automated enzyme assay (Olympus 20700 analyser (Olympus UK Ltd, Southall, UK)). Twolobes from each liver were fixed in 4% paraformaldehyde before routine histological processing. Histological grading of hepatic necrosis was performed by two blinded observers using H&E-stained sections as follows: <30% of the total area necrotic (1 point); 30–60% of the total area necrotic (2 points); >60% of the total area necrotic (3 points). Ten random fields were counted independently by two blinded observers to determine the number of apoptotic cells. Whole-liver homogenates were prepared and the Cell Death Detection ELISA (Roche, Welwyn Garden City, UK; quantitative detection of histone-associated DNA fragments in mononucleosomes and oligonucleosomes) was used as per the manufacturer’s instructions.

Protein analysis
Paraffin-wax-embedded sections were deparaffinised and subjected to microwave antigen retrieval in citrate buffer. The primary antibodies were rabbit polyclonal anti-phospho-JNK (Biosource, Nivelles, Belgium) and anti-paracetamol–protein adduct antibodies (HyCult Biotechnology, Uden, Netherlands). Species-appropriate isotype control antibodies were also used for each experiment. Western blot analysis was undertaken by using the following primary antibodies: rabbit polyclonal anti-phospho-JNK 1&2 [pTpY185/187] (Biosource), rabbit polyclonal anti-phospho-p38 antibody [pTpY190/192] (Biosource, Belgium) and rabbit polyclonal anti-β-actin antibody (Sigma, Gillingham, UK). JNK activity was measured as described previously.

Liver was homogenised in PBS with a protease inhibitor cocktail (Roche), and levels of immunoreactive tumour necrosis factor α (TNFα) and interferon γ (IFNγ) were measured in the supernatants by ELISA according to manufacturer’s instructions (R&D systems, Abingdon, UK).

Statistical analysis
Results are presented as means (SEM). Significance of the differences between means was assessed using one-way analysis of variance or two-tailed Student’s t test. Values of p<0.05 were considered significant. Unless stated otherwise, studies were performed on 3–6 independent occasions using 6–8 mice per group.

RESULTS
Paracetamol induces hepatic JNK activation in human and murine liver and precedes the onset of hepatocyte death
To determine whether JNK activity is increased during paracetamol-induced liver injury, we examined phospho-JNK expression. In a normal human liver, constitutive JNK phosphorylation occurs in areas surrounding the central veins (fig 1A). Cytoplasmic and dense nuclear staining was observed within hepatocytes. After paracetamol-induced ALF, there was a marked increase in hepatocyte cytoplasmic and nuclear phospho-JNK staining compared with the control normal liver (fig 1B; n=8 cases in each group). We also examined JNK activation in a murine model of paracetamol-induced hepatic injury. Phospho-JNK expression was negligible in the control mouse liver (fig 1C). Similar to human cases, paracetamol-injured mouse liver demonstrated marked upregulation of phospho-JNK in the hepatocyte nuclei and cytoplasm in the areas surrounding the central veins (fig 1D). The time course of JNK activation after paracetamol-induced injury was examined: hepatic JNK activity drastically increased after paracetamol administration, with peak activity 2 h after injection (fig 1E and inset). JNK activity then returned towards baseline levels by 24 h. Comparison with the time course of biochemical liver injury (determined by serum ALT) showed peak ALT 5 h after paracetamol injection (fig 1F).

Pharmacological inhibition of JNK activity in vivo markedly reduces mortality and liver injury
To assess the pathogenic significance of the observed increase in JNK activity, we used two pharmacological inhibitors of JNK that differ in their mechanisms of action. SP600125 is a small-molecule reversible ATP-competitive inhibitor, whereas D-JNKI1 is a peptide inhibitor that inhibits the interaction of JNK with its substrates. Immunohistochemistry for hepatic phospho-JNK demonstrated potent inhibition of JNK phosphorylation by SP600125 2 h after paracetamol treatment compared with control (fig 2A,B) and was confirmed by radioactive kinase assay (fig 2C) and western blotting for phospho-JNK (fig 2D). Importantly, pretreatment with SP600125 did not inhibit phosphorylation of the other major stress-activated protein kinase in the liver, p-38 (fig 2D). To determine the potential clinical significance of JNK activation after paracetamol poisoning, the JNK inhibitors (or control) were injected 1 h before paracetamol administration, and mortality was assessed over 72 h. Inhibition of JNK activity with either SP600125 or D-JNKI1 drastically reduced mortality compared with the vehicle control (fig 2E). In addition, sickness scores were assessed at 24 h, and the SP600125 and D-JNKI1 inhibitor groups showed significantly lower sickness scores than the vehicle control group (fig 2F).

To further evaluate the striking survival benefit of JNK inhibition after paracetamol administration, liver injury was assessed after JNK inhibition. SP600125 or D-JNKI1 dramatically reduced histological liver injury and hepatic necrosis scores compared with vehicle control (fig 3A,B). Furthermore, hepatocyte necrosis, as assessed by serum ALT release, was also reduced in the JNK inhibitor groups compared with vehicle control (fig 3C). Hepatocyte apoptosis may occur during paracetamol toxicity in the human and murine liver.
Further assessment of apoptosis by cell death ELISA using whole-liver homogenates also demonstrated a significant reduction in apoptosis in the JNK inhibitor groups 1, 2 and 5 h after paracetamol treatment compared with the control (fig 3F, p\(^{<0.01}\)).

Delayed administration of JNK inhibitor is more effective than NAC in limiting liver injury

An important clinical problem in the management of paracetamol poisoning is the group of patients who present >15 h after the overdose when the antidote, NAC, is much less effective. We have previously used NAC in a mouse model of paracetamol toxicity\(^{36}\) and demonstrated that beyond 5 h NAC is no longer effective in preventing liver injury. Therefore, we investigated whether delayed administration of the JNK inhibitor is more effective than NAC when given after a paracetamol overdose. Histological liver injury was less in the NAC and SP600125 groups (fig 4A). Furthermore, SP600125 was significantly more effective than NAC in limiting liver injury after paracetamol poisoning when given 5 h after an overdose, as assessed by hepatic necrosis scoring (fig 4B) and serum ALT release (fig 4C). However, JNK inhibition was no longer effective 8 and 24 h after paracetamol poisoning.

JNK inhibition is not protective in acute CCl\(_4\)-mediated or anti-Fas antibody-mediated hepatic injury

To determine whether JNK inhibition is protective in other forms of acute liver injury, we examined JNK inhibition in acute CCl\(_4\) and anti-Fas antibody-induced hepatic damage. Similar to paracetamol, CCl\(_4\) is a hepatotoxin metabolised in the liver by the cytochrome system, leading to the release of toxic free radicals and oxidant-mediated hepatic injury. Also, CCl\(_4\) injection increases JNK activity in the whole liver in vivo.\(^{37}\) To assess the effect of JNK inhibition in CCl\(_4\)-mediated liver injury, mice received CCl\(_4\) (at a ratio of 1:3 with olive oil at a dose of 1 \(\mu\)l/g body weight) 1 h after treatment with control, SP600125 or \(\alpha\)-JNKI1. Similar histological liver injury was observed in all groups (fig 5A), and there was no significant difference in hepatic necrosis scores (fig 5B) or biochemical liver injury at 24 h (fig 5C). Further experiments were also undertaken using CCl\(_4\) at a ratio of 1:3 with olive oil at a dose of 0.5 \(\mu\)l/g body weight, with no protection provided by JNK inhibition.

Previous studies have suggested that the Fas death pathway may play a role in the pathogenesis of paracetamol-induced liver injury.\(^{38-40}\) Therefore, we specifically examined the role of JNK inhibition in the anti-Fas antibody model of hepatic injury. Administration of anti-Fas (Jo-2) antibody directly ligates and...
Mice received anti-Fas... Therefore, we examined whether the JNK1... and JNK2 genes—

did not protect against paracetamol-induced liver injury

The JNK protein kinases are encoded by three genes. The liver expresses two of the three known JNK genes—JNK1 and JNK2.

**DISCUSSION**

We have shown a central role for JNK in the pathogenesis of paracetamol-induced ALF, thereby identifying JNK as a potential therapeutic target in the treatment of paracetamol hepatotoxicity. Our data indicated that: (1) paracetamol-induced liver injury results in hepatic JNK activation in human and murine tissue, which precedes the onset of hepatocyte death; (2) pharmacological inhibition of JNK in vivo markedly improved survival in a mouse model of paracetamol-induced ALF and decreased both hepatic necrosis and apoptosis. The protective effect of JNK inhibition was specific for paracetamol hepatotoxicity as the inhibitors were not protective in acute

To discuss in further detail the role of JNK1 and JNK2 in paracetamol-induced hepatic failure, we studied JNK1−/− and JNK2−/− mice (JNK1+2 double-null mice are embryonic lethal). Liver histology showed no protection in the JNK1−/− and JNK2−/− mice compared with WT mice (fig 6A), and no significant difference in hepatic necrosis scores (fig 6B) or biochemical liver injury between groups (fig 6C).

**JNK inhibition does not affect synthesis of paracetamol–protein adducts, but markedly inhibits TNFα production in the liver after paracetamol-induced hepatic injury**

Ingestion of paracetamol results in metabolism to NAPQI, which is effectively detoxified by conjugation with glutathione. However, in paracetamol poisoning, cytosolic and mitochondrial glutathione becomes depleted, allowing covalent binding of NAPQI to hepatocellular proteins (paracetamol–protein adducts). Although the exact mechanisms mediating paracetamol hepatotoxicity remain elusive, recent data have highlighted the important regulatory role of the immune system and cytokine networks in determining outcomes after paracetamol-induced liver injury. Therefore, we examined whether JNK inhibition interferes with the metabolism of paracetamol by measuring paracetamol–protein adducts. No paracetamol–protein adducts were seen in normal mouse liver (fig 7A). However, the formation of paracetamol–protein adducts (visible in hepatocytes around the central veins) was similar in all groups 5 h after paracetamol administration (vehicle control, SP600125 and α-JNK11 groups; fig 7B–D).

We also explored in vivo which inflammatory cytokines may be modulated by JNK inhibition in our model of paracetamol-induced liver injury. Previous studies have implicated interferon γ (IFNγ) in the pathogenesis of paracetamol-induced liver injury. Therefore, we examined whether the protective effect of JNK inhibition in paracetamol hepatotoxicity is mediated by modulation of TNFα expression. Hepatic TNFα levels were significantly increased in the vehicle control group 2 and 5 h after paracetamol administration compared with baseline (0 h). However, there was significantly less hepatic TNFα production in the JNK inhibitor groups 2 and 5 h after paracetamol administration compared with the vehicle control (fig 7E).

**NFκB** is an inflammatory cytokine that may play a significant pathogenic role in paracetamol hepatotoxicity. Although conflicting data have been reported. In vitro studies show that TNFα induces JNK activation in hepatocytes. In addition to the role of TNFα in apoptotic hepatocyte death, perhaps more relevant to paracetamol-induced liver injury, the JNK/AP-1 signalling pathway can also mediate TNFα-induced necrotic hepatocyte death. JNK activation can also induce TNFα expression via AP-1. Therefore, we examined whether the protective effect of JNK inhibition in paracetamol hepatotoxicity is mediated by modulation of TNFα expression. Hepatic TNFα levels were significantly increased in the vehicle control group 2 and 5 h after paracetamol administration compared with baseline (0 h). However, there was significantly less hepatic TNFα production in the JNK inhibitor groups 2 and 5 h after paracetamol administration compared with the vehicle control (fig 7F).

**ACTIVATION OF JNK**

activates the CD95 death receptor pathway, resulting in massive hepatocyte apoptosis within hours. Mice received anti-Fas antibody 1 h after treatment with control, SP600125 or α-JNK11. Similar histological liver injury was observed in all groups (fig 5D), and there was no significant difference in hepatic necrosis scores (fig 5E) or biochemical liver injury at 6 h (fig 5F). Further experiments were also undertaken using anti-Fas antibody at a dose of 0.25 mg/kg body weight, with no protection provided by JNK inhibition (data not shown).

**DISRUPTION OF THE JNK1 OR JNK2 GENES DOES NOT PROTECT AGAINST PARACETAMOL-INDUCED LIVER INJURY**

The JNK protein kinases are encoded by three genes. The liver expresses two of the three known JNK genes—JNK1 and JNK2.

**Figure 2** Pharmacological inhibition of c-jun (NH2) terminal kinase (JNK) activity in vivo markedly reduces mortality in a mouse model of paracetamol-induced acute liver failure. Mice were injected intraperitoneally (IP) with vehicle control or SP600125 (30 mg/kg) 1 h before IP injection with paracetamol solution (350 mg/kg). (A) Hepatic phospho-JNK (P-JNK) expression in the vehicle control group 2 h after paracetamol administration. (B) Hepatic P-JNK expression in the SP600125 group 2 h after paracetamol administration. (C) Time course of hepatic JNK activity in mice injected IP with vehicle control or SP600125 (30 mg/kg) 1 h before paracetamol administration (350 mg/kg). (D) Time course of hepatic P-JNK and phospho-p38 in mice injected IP with vehicle control or SP600125 (30 mg/kg) 1 h before paracetamol administration (350 mg/kg). (E) Mortality at 72 h in mice injected IP with vehicle control, SP600125 (30 mg/kg) or α-JNK11 (c-Jun N-terminal kinase inhibitor 1, α-stereoisomer; 30 mg/kg/mouse) 1 h before paracetamol administration (450 mg/kg; n = 10 mice in each group). (F) Sickness scores at 24 h in mice injected IP with vehicle control, SP600125 (30 mg/kg) or α-JNK11 (30 mg/kg/mouse) 1 h before paracetamol administration (350 mg/kg/mouse; n = 10 mice in each group). Each point represents an individual mouse.

**Figure 2A** Control+paracetamol

**Figure 2B** SP600125+paracetamol

**Figure 2C** P-c-jun

**Figure 2D** P-JNK

**Figure 2E** Morality %

**Figure 2F** Sickness score
Figure 3 c-Jun (NH2) terminal kinase (JNK) inhibition in vivo reduces hepatic necrosis and apoptosis in paracetamol-induced acute liver failure. Mice were injected intraperitoneally (IP) with vehicle control, SP600125 (30 mg/kg) or β-JNKI1 (c-jun N-terminal kinase peptide inhibitor 1, β-stereoisomer; 30 µg/mouse) 1 h before paracetamol administration (350 mg/kg; n = 6 mice in each group). (A) Liver histology at 24 h after paracetamol. Scale bar 200 µm. (B) Hepatic necrosis scores at 24 h after paracetamol administration (*p<0.001). (C) Serum alanine aminotransferase (ALT) release at 24 h after paracetamol administration (**p<0.05). (D) Liver histology at high magnification (>×200) 5 h after paracetamol administration demonstrating apoptotic cells (arrowed) in the control vehicle-treated group. (E) Time course of apoptotic cells/high power field (HPF; ***p<0.01). (F) Time course of apoptosis assessed by cell death ELISA (quantitative detection of histone-associated DNA fragments in mononucleosomes and oligonucleosomes) from whole-liver homogenates (**p<0.01).

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Figure 4 Delayed administration of c-jun (NH2) terminal kinase (JNK) inhibitor is more effective than N-acetylcysteine (NAC) in limiting liver injury after paracetamol poisoning in mice. Mice were treated with paracetamol (350 mg/kg intraperitoneally) and 5 h later administered control vehicle, NAC or SP600125 (30 mg/kg; n = 6 mice in each group). (A) Liver histology at 24 h after paracetamol administration. Scale bar 200 µm. (B) Hepatic necrosis scores at 24 h after paracetamol administration (*p<0.05). (C) Serum alanine aminotransferase (ALT) release 24 h after paracetamol administration (*p<0.05).

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CCl₄-mediated or anti-Fas antibody-mediated hepatic injury; (3) delayed administration of JNK inhibitor is more effective than NAC in limiting liver injury in mice; (4) disruption of either the JNK1 or JNK2 genes was not protective; and (5) inhibition of the JNK had no effect on paracetamol metabolism, but inhibited hepatic TNFα production after paracetamol-induced ALF.

Paracetamol poisoning is the most common cause of ALF in the UK and USA, causing significant morbidity and mortality in predominantly young people. Many patients present outside the therapeutic window for the commonly used antidote, NAC. Our data demonstrated that delayed administration of the JNK inhibitor is more effective than NAC after paracetamol poisoning in mice. Although NAC can be administered up to 15 h after a paracetamol overdose in clinical practice, we found limited efficacy 5 h after paracetamol administration in the mouse model. This is in keeping with our previously published data, and relates to the truncated time course of liver injury in mice compared with humans. Metabolic activation of paracetamol and the histological liver injury produced in mice is similar to that observed in humans; however, the variability in terms of the clinical context in which the paracetamol overdose is taken and the genetic background of humans limits direct extrapolations of our results to the treatment of patients. Despite these limitations, our data indicate that further studies of JNK activation in cases of paracetamol poisoning in humans may lead to the development of new treatments and that pharmacological inhibition of JNK may be of particular clinical use in patients with delayed presentation after paracetamol overdose.

Our data show prolonged hepatic activation of JNK after paracetamol overdose in both the human and murine liver. Interestingly, in the normal human liver phospho-JNK staining was observed in hepatocytes surrounding the central veins, the area in which paracetamol is preferentially activated by the hepatic cytochrome P450 system. Hepatic JNK activation may be a consequence of the oxidative stress produced during paracetamol metabolism. ROS are widely recognised to induce increased and/or prolonged JNK activation, possibly because of inactivation of cellular phosphatases. Although ROS alone can activate JNK, hepatic TNFα expression is increased in paracetamol-induced liver injury. TNFα is a potent inducer of

Figure 5  c-Jun (NH2) terminal kinase (JNK) inhibition is not protective in acute carbon tetrachloride (CCl₄)- or anti-Fas antibody-mediated hepatic injury. CCl₄ model: mice were administered CCl₄ at a ratio of 1:3 with olive oil at a dose of 1 μl/g body weight 1 h after treatment with control, SP600125 (30 mg/kg) or D-JNK11 (c-Jun N-terminal kinase peptide inhibitor 1, d-stereoisomer; 30 μg/mouse; n = 6 mice in each group). (A) Liver histology 24 h after CCl₄ administration. Scale bar 200 μm. (B) Hepatic necrosis scores 24 h after CCl₄ administration (p = NS). (C) Serum alanine aminotransferase (ALT) release 24 h after CCl₄-induced liver injury (p = NS). Fas model: mice were administered anti-Fas antibody (0.5 μg/g body weight) 1 hour after treatment with control, SP600125 (30 mg/kg) or D-JNK11 (30 μg/mouse; n = 6 mice in each group). (D) Liver histology 6 h after anti-Fas antibody administration. Scale bar 200 μm. (E) Hepatic necrosis scores 6 h after anti-Fas antibody administration (p = NS). (F) ALT release 6 h after anti-Fas antibody-induced liver injury (p = NS).
both JNK and ROS. Therefore, in the hepatic cytokine milieu induced by paracetamol, a positive amplification loop may exist whereby paracetamol-induced ROS leads to JNK activation and TNFα expression via AP-1, resulting in further prolonged massive JNK activation in the liver via TNF receptor signalling.

To investigate the potential pathogenic role of JNK activation in the development of paracetamol-induced hepatic necrosis, we used two different JNK inhibitors with different mechanisms of action (SP600125 and D-JNKI1). Both inhibitors have been widely studied both in vivo and in vitro. Although the specificity of pharmacological inhibitors can be questioned, we found no reduction in the hepatic activation of another stress-activated protein kinase, p38, after administration of the JNK inhibitor and paracetamol. Furthermore, despite different mechanisms of action, both JNK inhibitors conferred significant survival benefit and profound protection against paracetamol-induced hepatic necrosis.

Several studies have shown the mechanistic importance of the hepatic cytokine network in paracetamol-induced liver injury. To investigate the mechanisms underlying the protective effect of JNK inhibition in paracetamol hepatotoxicity, we measured the hepatic expression of IFNγ and TNFα. Both these cytokines are induced in the liver after paracetamol poisoning. Furthermore, IFNγ knockout mice are resistant to paracetamol-induced liver injury. However, in our model, JNK inhibition had no significant effect on hepatic IFNγ. By contrast, JNK inhibition significantly reduced hepatic TNFα expression. We and others have shown increased TNFα expression in the peripheral circulation and liver after paracetamol poisoning. In the studies reporting a protective effect of TNFα inhibition in paracetamol-induced liver injury, the degree of protection was similar to that observed with JNK inhibition in our study. However, the pathogenic role of TNFα in paracetamol-induced hepatic necrosis remains controversial. Studies using blocking antibodies and inhibitors of TNFα have produced conflicting results, with both protection and no effect being reported. Alternatively, some have shown attenuated liver injury in mice lacking TNF receptor 1 (TNFR1) expression, while others, using a different strain of mice, have shown increased liver injury in TNFR1 knockout mice. Our data suggest that JNK inhibition may limit liver injury via reduced TNFα expression, although in view of the
This study reported that Jones BE. In contrast with our data, the mammalian UV response: c-Jun expression. From a clinical standpoint, we have shown that JNK inhibition is more efficacious in reducing liver injury at later time points when the traditional antidote NAC is no longer effective. These data demonstrate that JNK plays a crucial role in hepatocyte death after paracetamol poisoning, and suggest that JNK inhibition may find clinical applications in patients who present late after overdose or in whom the timing of the overdose is unclear.

ACKNOWLEDGMENTS

We thank Kirsten Atkinson and Anne Pryde for expert technical assistance. This work was supported by the Scottish Health Endowments Research Trust (SHERT; Grant Number RG56/01), the Wellcome Trust, UK and a Lothian University Hospitals NHS Trust Research and Development grant (Grant Number PG2004/26).

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Competing interests: None.

REFERENCES


