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A small-molecule inhibitor of NF-κB-inducing kinase (NIK) protects liver from toxin-induced inflammation, oxidative stress, and injury

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ABSTRACT: Potent and selective chemical probes are valuable tools for discovery of novel treatments for human diseases. NF-κB-inducing kinase (NIK) is a key trigger in the development of liver injury and fibrosis. Whether inhibition of NIK activity by chemical probes ameliorates liver inflammation and injury is largely unknown. In this study, a small-molecule inhibitor of NIK, B022, was found to be a potent and selective chemical probe for liver inflammation and injury. B022 inhibited the NIK signaling pathway, including NIK-induced p100-to-p52 processing and inflammatory gene expression, both *in vitro* and *in vivo*. Furthermore, *in vivo* administration of B022 protected against not only NIK but also CCl_4-induced liver inflammation and injury. Our data suggest that inhibition of NIK is a novel strategy for treatment of liver inflammation, oxidative stress, and injury.—Ren, X., Li, X., Jia, L., Chen, D., Hou, H., Rui, L., Zhao, Y., Chen, Z. A small-molecule inhibitor of NF-κB-inducing kinase (NIK) protects liver from toxin-induced inflammation, oxidative stress, and injury. FASEB J. 31, 711–718 (2017). www.fasebj.org

KEY WORDS: NIK · liver injury · inflammation · CCl_4

Liver injury represents a growing major health concern worldwide. Specifically, toxin-induced liver injury has dramatically increased in both developed and developing countries. Toxins [*e.g.*, drugs, herbals, and carbon tetrachloride (CCl_4)] cause hepatocyte damage by increasing permeability of cellular membranes (1), concentrations of highly reactive free radicals (2), and expression of proinflammatory cytokines (*e.g.*, TNF-α and IL-6) (3, 4), eventually resulting in severe apoptosis and necrosis (5, 6). The detailed molecular mechanisms of toxin-induced liver injury are not fully understood. It has not been completely demonstrated whether selective chemical probes can be valuable tools for the treatment of liver injury.

Recently, the noncanonical NF-κB signaling pathway has been shown to play an important role in the development of liver injury and fibrosis (7, 8). The critical event in this signaling pathway is the accumulation of NF-κB-inducing kinase (NIK), which phosphorylates IKKα and NF-κB2, leading to p100-to-p52 processing (9–11). p52 is the mature form of NF-κB2, which triggers the expression of inflammatory genes (9, 10, 12). In patients with cirrhosis, the levels of both *NIK* mRNA and p52 protein are significantly increased. In a CCl_4- or alcohol-induced liver injury mouse model, *NIK* mRNA and p52 protein levels are also abnormally augmented. Liver-specific overexpression of NIK induces hepatocytes to secrete proinflammatory cytokines that cause liver inflammation and destruction, leading to death in mice (7). Overall, NIK activation is prone to cause liver inflammation and injury.

Given the strong association between NIK activation and liver injury, inhibition of NIK or the NF-κB2 signaling pathway represents attractive therapeutic strategies for the treatment of liver inflammation and injury. A small molecule, B022, has been reported to be a potent inhibitor of NIK activity, with K_i = 4.2 nM in an ATP-consumption assay (13). A derivative of B022, AM-0216 in the low micromolar range has exhibited NIK-dependent cytotoxicity in myeloma cells (14). However, whether inhibition of NIK

ABBREVIATIONS: β-Gal, β-galactosidase; ALT, alanine aminotransferase; NIK, NF-κB-inducing kinase; qPCR, quantitative PCR; ROS, reactive oxygen species; WT, wild-type

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by B022 ameliorates liver inflammation and injury is largely unknown.

In this study, B022 suppressed NIK-induced liver inflammation and injury. It decreased NIK-induced p100-to-p52 processing and inflammatory gene expression in hepatocytes. Intravenous administration of B022 in mice ameliorated both NIK- and CCl_4-induced liver inflammation and injury. These observations indicate that inhibition of NIK activity by small molecules could be a novel strategy for the treatment of liver inflammation and injury.

MATERIALS AND METHODS

Animal experiments

Animal experiments were performed in strict accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, Bethesda, MD, USA), and were approved by the Institutional Animal Care and Use Committee or Animal Experimental Ethics Committee of Northeast Normal University. *STOP-NIK* mice (C57BL/6 background) are described in Shen *et al.* (7) and Jiang *et al.* (8). *STOP-NIK* male mice (8 wk) were infected with *Ad-cre* (2×10^{11} viral particles per mouse) *via* tail vein injection and then were injected with B022 (30 mg/kg bodyweight) or an equal volume of vehicle *via* tail vein twice a day for 10 d. For the CCl_4-induced liver injury mouse model, wild-type (WT) C57BL/6 WT mice were intraperitoneally injected with CCl_4 (2.5 ml/kg bodyweight, 10% in olive oil) and then injected with B022 (25 mg/kg bodyweight) or an equal volume of vehicle *via* tail vein at 0, 3, 6, and 9 h after CCl_4 injection. The mice were euthanized at 12 h after CCl_4 injection. Blood samples were collected from the retro-orbital sinus. Blood glucose levels were determined with glucometers (Beijing Yicheng Bioelectronics Technology Co., Beijing, China). The plasma alanine aminotransferase (ALT) activity level was measured with an ALT reagent set.

Chemical synthesis of the NIK inhibitor B022

The synthesis of B022 was performed based on a reported procedure, with modifications (15). The synthetic route and characterization data are provided in the Supplemental Data.

Cell culture and adenoviral infection

HEK293 and Hepa1 cells were grown at 37°C in 5% CO_2 in DMEM supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum. Primary hepatocytes were isolated from C57BL/6 WT mice (16, 17). Hepa1 cells and primary hepatocytes were then infected with adenoviruses (8, 18).

Treatment of RAW cells with hepatocyte-conditioned medium

Primary hepatocytes were infected with β-galactosidase (β-Gal), infected with NIK adenovirus, or infected with NIK adenovirus and treated with B022 (10 μM) for 18 h. β-Gal and NIK adenovirus–infected groups were then grown in fresh growth medium. The groups of NIK adenovirus-infected cells treated with B022 (10 μM) were then cultured in fresh growth medium containing B022 (10 μM). Hepatocyte-conditioned medium was collected 24 h later (7). RAW cells were treated with 50% hepatocyte-conditioned medium for 12 h. Gene expression was measured by quantitative PCR (qPCR).

Transient transfection and luciferase assays

HEK293 cells were divided equally in a 24-well plate and cultured for 20 h, and then cotransfected with NF-κB luciferase reporter plasmids with NIK or an empty expression vector *via* polyethylenimine (Sigma-Aldrich, St. Louis, MO, USA). Twelve hours later, the HEK293 cells were incubated with different doses of B022 (0, 0.5, or 5 μM) for another 36 h. The cells were lysed in reporter lysis buffer (Promega, Madison, WI, USA) 48 h after transfection, and luciferase activity was measured and normalized to β-Gal activity (19).

Immunoblot analysis

Immunoblot analysis has been described in several publications (17, 20, 21). In brief, cells or tissues were homogenized in an L-RIPA lysis buffer [50 mM Tris (pH 7.5), 1% Nonidet P-40, 150 mM NaCl, 2 mM EGTA, 1 mM Na_3VO_4, 100 mM NaF, 10 mM $Na_4P_2O_7$, and 1 mM PMSF]. Protein was separated by SDS-PAGE, immunoblotted with the indicated antibodies, and visualized by ECL. Antibody dilution ratios were as follows: Flag (F1804, 1:4000; Sigma-Aldrich), NF-κB2 (4882, 1:4000; Cell Signaling Technology, Danvers, MA, USA), tubulin (sc5286, 1:5000; Santa Cruz Biotechnology, Dallas TX, USA), and lamin B1 (12987-1-AP, 1:5000; Proteintech, Chicago, IL, USA).

Reactive oxygen species assays

Reactive oxygen species (ROS) assays have been described (7). In brief, liver samples were homogenized in L-RIPA buffer. Liver

TABLE 1. *Primers for qPCR*

Genes	Primer, 5′–3′	
	Forward	Reverse
TNFα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
IL6	AGCCAGAGTCCTTCAGA	GGTCCTTAGCCACTCCT
IL1β	GCCTTGGGCCTCAAAGGAAAGAATC	GGAAGACACAGATTCCATGGTGAAG
iNOS	CAGGGCCACCTCTACATTTG	TGCCCCATAGGAAAAGACTG
NIK	TCTCTGGAGGAACAGGAACAA	GCCATTGAGAGACTGGATCTG
36B4	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
CCL2	ACTGAAGCCAGCTCTCTCTTCCTC	TTCCTTCTTGGGGTCAGCACAGAC
CCL5	CCACTTCTTCTCTGGGTTGG	GTGCCCACGTCAAGGAGTAT
CXCL5	TGCATTCCGCTTAGCTTTCT	CAGAAGGAGGTCTGTCTGGA
p100	CCACCAGCCAGCTTGTAA	TGCTGCTAAATGCTGCTCA

extracts were incubated with dichlorofluorescein diacetate fluorescent (final concentration, 5 μM) probes (D6883; Sigma-Aldrich) at 37°C for 1 h. Fluorescence was measured with an Infinite 200 Pro microplate reader (Tecan, Männedorf, Switzerland) and normalized to tissue weight.

Real-time qPCR

Total RNAs were extracted using TriPure Isolation Reagent (Roche Diagnostics, Mannheim, Germany), and the first-strand cDNAs were synthesized with random primers and M-MLV reverse transcriptase (Promega) (16). RNA abundance was measured with qPCR SYBR Mix and a LightCycler 480 real-time PCR system (both from Roche Diagnostics). The expression of individual genes was normalized to the expression of 36B4, a housekeeping gene. Primers for real-time qPCR were listed in **Table 1**.

Statistical analysis

Data are presented as means ± SE. Differences between groups were analyzed by Student's *t* tests, with $P < 0.05$ indicating statistical significance.

RESULTS

B022 suppresses NIK/NF-κB2 signaling

To determine whether B022 inhibits NIK-induced p100-to-p52 processing in hepatocytes, Hepa1 cells were infected with p100 and NIK adenoviruses and treated with B022 at different doses. NIK overexpression dramatically promoted p100-to-p52 processing, as judged by immunoblot assay. Furthermore, B022 suppressed NIK-induced p52 formation in a dose-dependent manner (**Fig. 1*A***).

We then measured NF-κB2 activity by using an NF-κB luciferase reporter assay. The NF-κB luciferase reporter plasmid was transiently transfected into HEK293 cells, together with an NIK expression plasmid. These cells were subsequently treated with B022 at different doses, and luciferase activity was measured 48 h after transfection. NF-κB luciferase reporters are expected to be activated by endogenous NF-κB2. NIK markedly increased NF-κB luciferase reporter activity, which was suppressed by B022 dose dependently (Fig. 1*B*). In the absence of NIK, B022 alone did not alter basal NF-κB luciferase reporter activity. Taken together, these data suggest that B022 suppresses NIK-induced activation of endogenous NF-κB2.

B022 blocks NIK-induced inflammatory gene expression in hepatocytes

To determine whether B022 inhibits NIK-induced expression of inflammatory genes in hepatocytes, Hepa1 cells or primary hepatocytes were infected with NIK adenoviruses, and then treated with B022 at different doses. Gene expression was measured by qPCR. Compared with β-Gal, NIK largely triggered the expression of liver chemokines (*e.g.*, CCL2, CCL5, and CXCL5), proinflammatory cytokines (TNF-α and IL-6), and iNOS

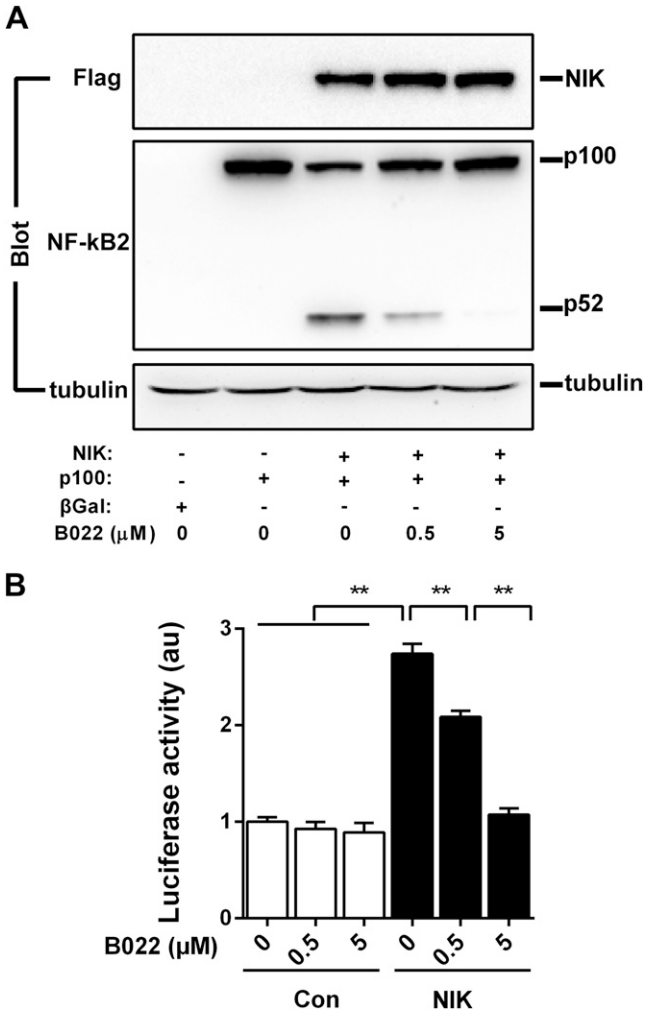

Figure 1. B022 suppresses NIK-stimulated activation of the noncanonical NF-κB2 signaling pathway. *A*) Hepa1 cells were infected with p100 and Flag-tagged NIK adenovirus in the presence of B022 at different doses (0, 0.5, or 5 μM). Cell extracts were prepared 12 h after B022 treatment and immunoblotted with antibodies to NF-κB2, Flag, or tubulin. *B*) NF-κB luciferase reporter and NIK plasmids were cotransfected into HEK293 cells. Twelve hours after transfection, HEK293 cells were treated with B022 at different doses (0, 0.5, or 5 μM) for another 36 h. Luciferase activity was then measured and normalized to β-Gal activity ($n = 4$). $^{**}P < 0.01$. Au, arbitrary unit.

(**Fig. 2**; Supplemental Fig. S1). In addition, B022 dose-dependently blocked NIK-induced expression of chemokines, cytokines, and iNOS in these cells.

To further determine the time course of the inhibitory effect of B022 on gene expression in hepatocytes, Hepa1 cells were infected with NIK adenovirus, and then treated with B022 (5 μM) for different durations (0, 1, 2, 4, or 8 h). Treatment with B022 for 1 h was sufficient to inhibit NIK-induced TNF-α expression (**Fig. 3*A***). B022 treatment for 8 h completely blocked NIK-induced expression of TNF-α, IL-6, iNOS, CCL2, and CXCL5 (Fig.3*A–D*, *F*). NIK-induced expression of CCL5 was also decreased after B022 treatment for 8 h (Fig. 3*E*). These data indicate that B022 acts quickly to inhibit NIK activity in Hepa1 cells.

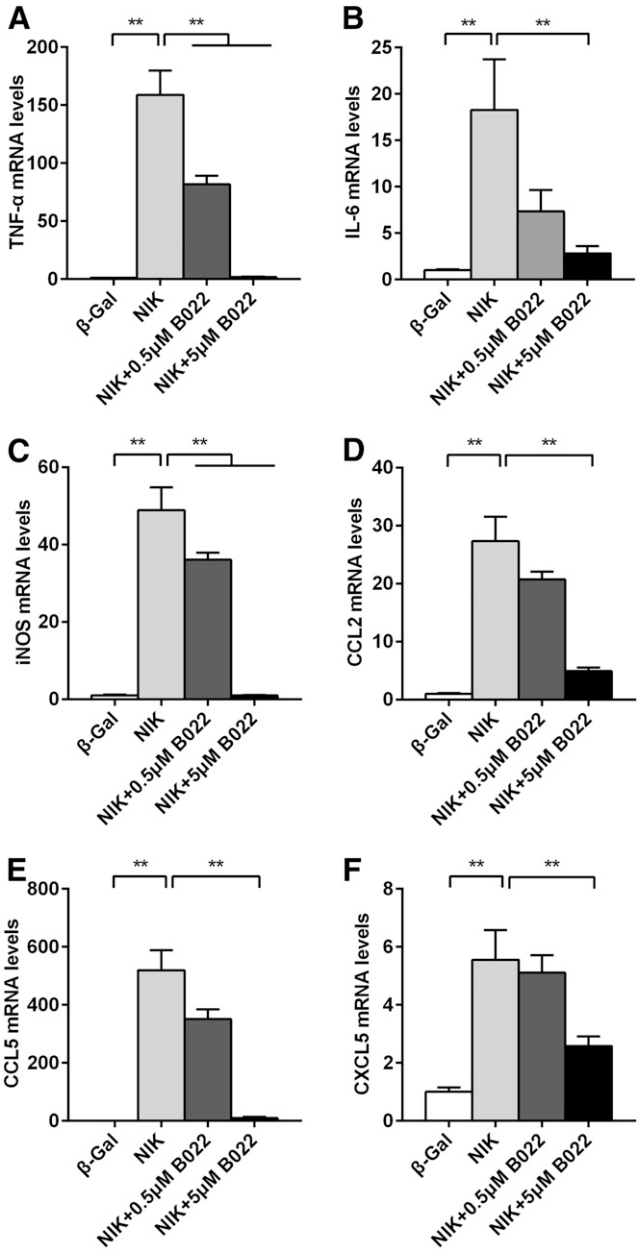

Figure 2. B022 dose-dependently blocks NIK-stimulated expression of inflammatory factors. Hepa1 cells were infected with NIK adenovirus in the presence of B022 at different doses (0, 0.5, or 5 μM) for 12 h. The expression of TNF-α (*A*), IL-6 (*B*), iNOS (*C*), CCL2 (*D*), CCL5 (*E*), and CXCL5 (*F*) was measured by qPCR ($n = 6$). $^{**}P < 0.01$.

B022 inhibits NIK-triggered liver inflammation and injury

To test whether B022 suppresses NIK action in animals, we employed *STOP-NIK* mice, in which a *STOP-NIK* cassette is knocked into the *Rosa26* locus. Cre-mediated deletion of the *STOP* sequences activates *NIK* overexpression (7, 8). We injected purified *cre* adenoviruses, which express Cre under the control of the *CMV* promoter, to generate liver-specific NIK transgenic mice (7, 8). Liver *NIK* mRNA levels were much higher in the *cre* group than in the control group (**Fig. 4*A***). *p100* mRNA levels were also significantly increased (Fig. 4*B*), and p52 NF-κB2 protein levels were dramatically increased (17-fold) in the *cre* group than in the control group (Fig. 4*C*). Consistent with the *in vitro* assays, intravenous administration of B022 in the *cre* group decreased the levels of the mature form of p52 NF-κB2 by ~62% (Fig. 4*C*) without affecting *NIK* and *p100* mRNA levels (Fig. 4*A*, *B*) in the liver, presumably because of direct inhibition of NIK activity.

It has been reported that hepatocyte-specific activation of the *NIK* transgene results in liver inflammation and injury, leading to death in mice (7, 8). To determine whether B022 attenuates the detrimental effect of NIK in the liver, *STOP-NIK* mice were infected with *cre* adenovirus *via* tail vein injection, and then B022 or vehicle was injected *via* tail vein twice a day for 10 d. Liver-specific activation of the *NIK* transgene caused death of *STOP-NIK* mice within 10 d after infection with *cre* adenovirus, and intravenous administration of B022 completely rescued them from NIK-induced death (Fig. 4*D*). These data demonstrate that B022 completely prevents the lethal effect of abnormally high levels of hepatic NIK in mice.

Liver-specific activation of NIK causes liver injury, as revealed by decreased blood glucose levels, increased blood ALT activity, liver weight, liver inflammation, and liver oxidative stress in *STOP-NIK* mice infected with *cre* adenoviruses (7). Intravenous administration of B022 largely prevented these harmful effects of aberrant hepatic NIK in *STOP-NIK* mice infected with *cre* adenovirus, as revealed by significantly increased blood glucose levels (Fig. 4*E*), decreased blood ALT activity (Fig. 4*F*), decreased liver weight (Fig. 4*G*), reduced immune cell infiltration into the liver (Fig. 4*H*), decreased levels of reactive oxygen species (Fig. 4*I*), reduced TUNEL$^+$ cells (Fig. 4*J*), and decreased expression of proinflammatory genes (Fig. 4*K*). Together, these results demonstrate that B022 inhibits the majority of the deteriorating effects of aberrant activation of hepatic NIK.

Hepatocyte-specific overexpression of NIK has been shown to trigger liver injury and fibrosis, which is associated with macrophages (7). We determined whether B022 affects the crosstalk between hepatocytes and macrophages. Primary hepatocytes were infected with β-Gal, NIK adenovirus, or NIK adenovirus plus B022, and the hepatocyte-conditioned media were collected. RAW cells were treated with each conditioned medium. NIK adenovirus plus treatment with B022 hepatocyte-conditioned medium significantly suppressed the expression of iNOS and IL-1β in RAW cells (Supplemental Fig. S2), indicating that B022 may also prevent macrophage-induced inflammation in liver injury.

B022 ameliorates CCl_4-induced acute liver inflammation and injury

To further determine whether B022 ameliorates CCl_4-induced liver inflammation and injury in mice, WT mice were intraperitoneally injected with CCl_4, and B022 or vehicle was injected *via* the tail vein every 3 h. CCl_4 increases the *NIK* mRNA and p52 NF-κB2 levels (7). In agreement, liver *NIK* and *p100* mRNAs were much higher in the CCl_4-injected mice than in the control mice (**Fig. 5*A*, *B***). p52 NF-κB2 protein levels increased by 2.1-fold in the CCl_4-injected mice compared with those in the control mice (Fig. 5*C*), whereas intravenous administration of

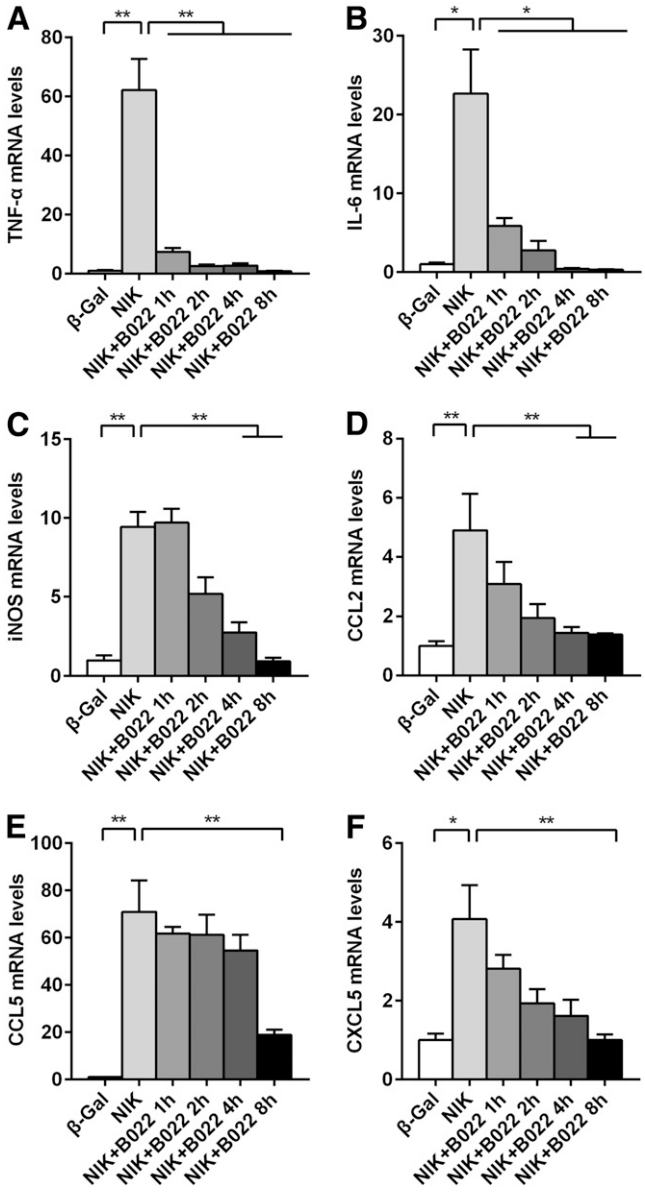

Figure 3. B022 time-dependently inhibits NIK-stimulated expression of inflammatory factors. Hepa1 cells were infected with NIK adenovirus in the presence of B022 (5 μM) for different times (0, 1, 2, 4, or 8 h). The expression of TNF-α (*A*), IL-6 (*B*), iNOS (*C*), CCL2 (*D*), CCL5 (*E*), and CXCL5 (*F*) was measured by qPCR ($n = 4$). $^*P < 0.05$; $^{**}P < 0.01$.

B022 in CCl_4-injected mice decreased these levels by ~73% in the liver without affecting *NIK* and *p100* mRNA levels (Fig. 5*A*–5*C*). These results further confirm the specificity of NIK inhibition by B022. Moreover, B022 injection significantly decreased blood ALT activity (Fig. 5*D*), immune cell infiltration into the liver (Fig. 5*E*), levels of ROS (Fig. 5*F*), and the number of $TUNEL^+$ cells (Fig. 5*G*). CCl_4 injection significantly increased expression of proinflammatory genes (Fig. 5*H–M*), while treatment with B022 also dramatically decreased expression of proinflammatory genes (Fig. 5*H–K, M*). Together, these results demonstrate that B022 ameliorates CCl_4-induced liver inflammation and injury, indicating that inhibition of NIK activity by a small molecule could be a novel strategy for treatment of liver inflammation and injury.

DISCUSSION

Liver injury and chronic inflammation are two major causes of liver fibrosis and cirrhosis that lead to mortality and morbidity. JNK, IKK/NF-κB, and TGF-β signaling pathways have been shown to promote liver injury, inflammation, and fibrosis (22). Inhibition of JNK by its inhibitor SP600125 ameliorates both CCl_4 and bile duct ligation-induced liver fibrosis (23). Pirfenidone, an inhibitor of TGF-β production, has been evaluated in a small study in HCV-related fibrosis (24) and has been shown to improve long-term liver inflammation and fibrosis (24, 25). IFN-γ, an endogenous antagonist of TGF-β, has been shown to reduce the expression of type I and IV collagen, fibronectin, and SMA (26). IFN-β1b is currently in a phase II study in patients with liver fibrosis and cirrhosis (NCT00043303; *https://clinicaltrials.gov*) (25).

Recently, NIK/p52 NF-κB2 has been shown to be a key trigger for liver injury (7, 8). Hepatic NIK is aberrantly activated in multiple liver diseases, including alcoholic liver disease, drug-induced liver disease, and primary biliary cirrhosis (7). In mice, hepatocyte-specific overexpression of NIK is sufficient to cause severe liver inflammation and injury, leading to death within a few weeks (7). Several small molecules have been reported to be inhibitors of NIK activity in an ATP consumption assay (13). In this study, we tested whether inhibition of NIK activity by one of these small molecules (B022) could ameliorate liver inflammation and injury.

In our study, B022 directly inhibited NIK activity in Hepa1 cells and primary hepatocytes. It inhibited NIK-induced p100-to-p52 processing, and the expression of inflammatory cytokines and *iNOS* genes. Moreover, we observed *in vivo* that B022 completely reversed NIK-induced hepatocyte death and largely corrected the abnormalities caused by hepatocyte-specific overexpression of NIK (*e.g.*, loss of bodyweight, hypoglycemia, liver inflammation, liver oxidative stress, and hepatomegaly). These findings demonstrate that B022 specifically suppresses the abnormal activation of NIK, thus protecting against liver injury.

Toxins (CCl_4) cause hepatocyte death by increasing permeability of cellular membranes (1), concentrations of highly reactive free radicals (2), and expression of proinflammatory cytokines (TNF-α and IL-6) (3, 4). It has been shown that NIK/p52 NF-κB2 is strongly associated with toxin (CCl_4)-induced liver injury (7). In our study, the expression of NIK/p52 NF-κB2 was dramatically increased in CCl_4-induced liver injury. Inhibition of NIK by B022 completely blocked CCl_4-induced nuclear p52 protein levels in the liver. B022 injection also significantly reduced blood ALT activity, number of $TUNEL^+$ cells, and expression of proinflammatory genes. Moreover, B022 injection completely reversed CCl_4-induced *iNOS* gene expression and ROS levels in the liver. These observations indicate that B022 ameliorates CCl_4-induced liver injury primarily through the inhibition of p52 and ROS formation.

NIK- or CCl_4-induced liver injury and fibrosis are strongly associated with macrophage-induced inflammation (7). Conditioned medium from NIK-overexpressing hepatocytes dramatically triggered RAW cells to express *iNOS* and *IL-1β*, but B022 treatment significantly suppressed

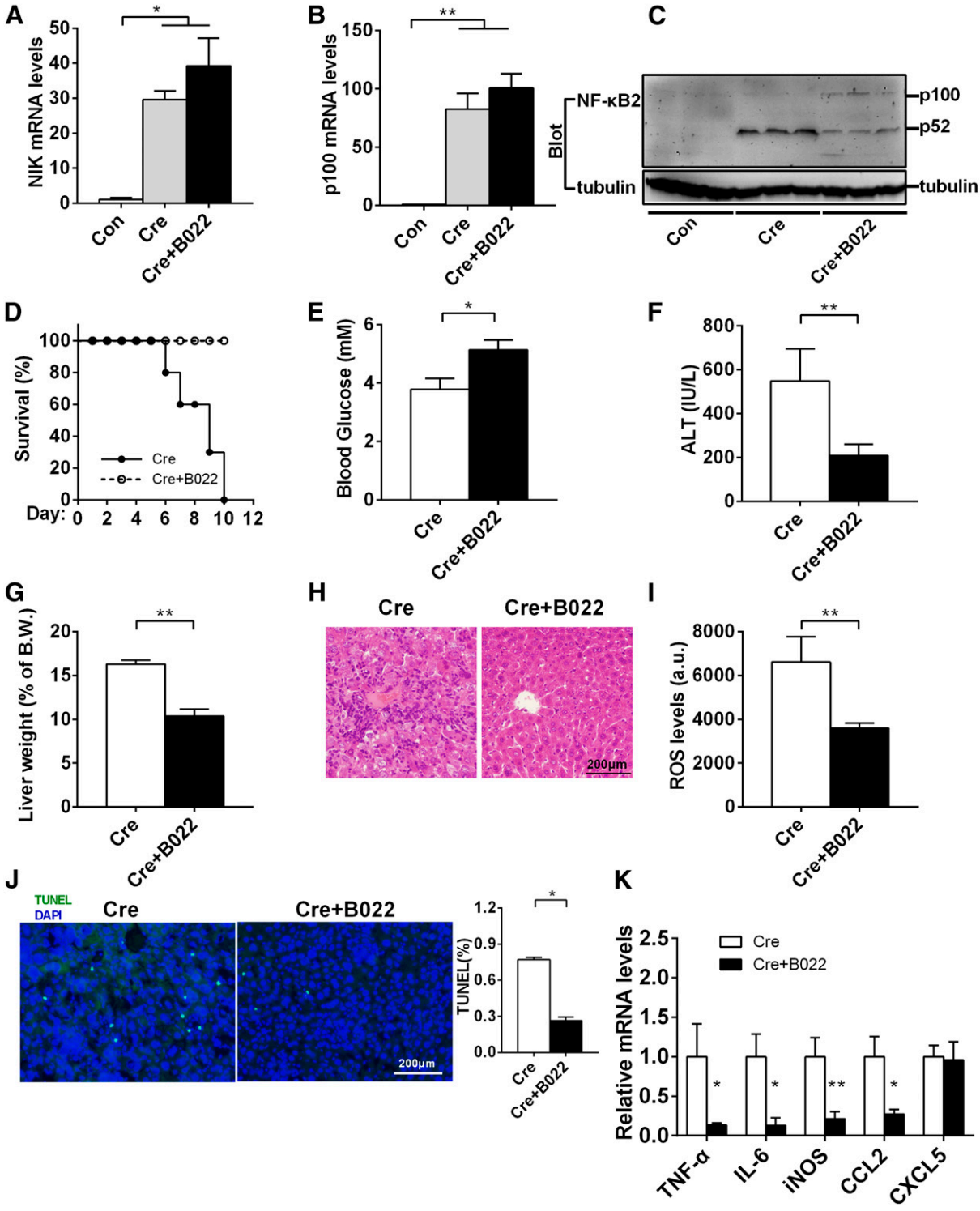

Figure 4. B022 suppresses NIK-triggered liver inflammation and injury. *STOP-NIK* male mice (8 wk) were infected with *Ad-cre* (2×10^{11} viral particles per mouse) and *ad-GFP via* tail vein injection. *Cre*-injected mice were then injected with B022 (30 mg/kg bodyweight) or an equal volume of vehicle *via* the tail vein twice a day for 10 d. *A*, *B*) Liver *NIK* (*A*) and *p100* (*B*) mRNA levels were measured by qPCR and normalized to 36B4 levels. *C*) Liver extracts were prepared and immunoblotted with antibodies to NF-κB2 or tubulin. *D*–*J*) Survival curves (*D*), blood glucose levels (*E*), blood ALT activity (*F*), liver weight (% bodyweight) (*G*), H&E staining (*H*), liver reactive oxygen species (ROS) levels (*I*), and TUNEL staining of frozen liver sections (*J*). *K*) Liver mRNA abundance was measured by qPCR and normalized to 36B4 levels. $n = 3$–9. $^*P < 0.05$; $^{**}P < 0.01$.

those effects. This finding indicates that B022 may also prevent macrophage-induced inflammation in liver injury. The therapeutic role of B022 in macrophage-induced inflammation and related diseases should be further examined.

It has been reported that the *in vivo* pharmacokinetic properties of this class of NIK inhibitor are not optimal. In line with another report (14), multiple tandem, high doses of B022 are necessary to achieve *in vivo* inhibition of NIK- or

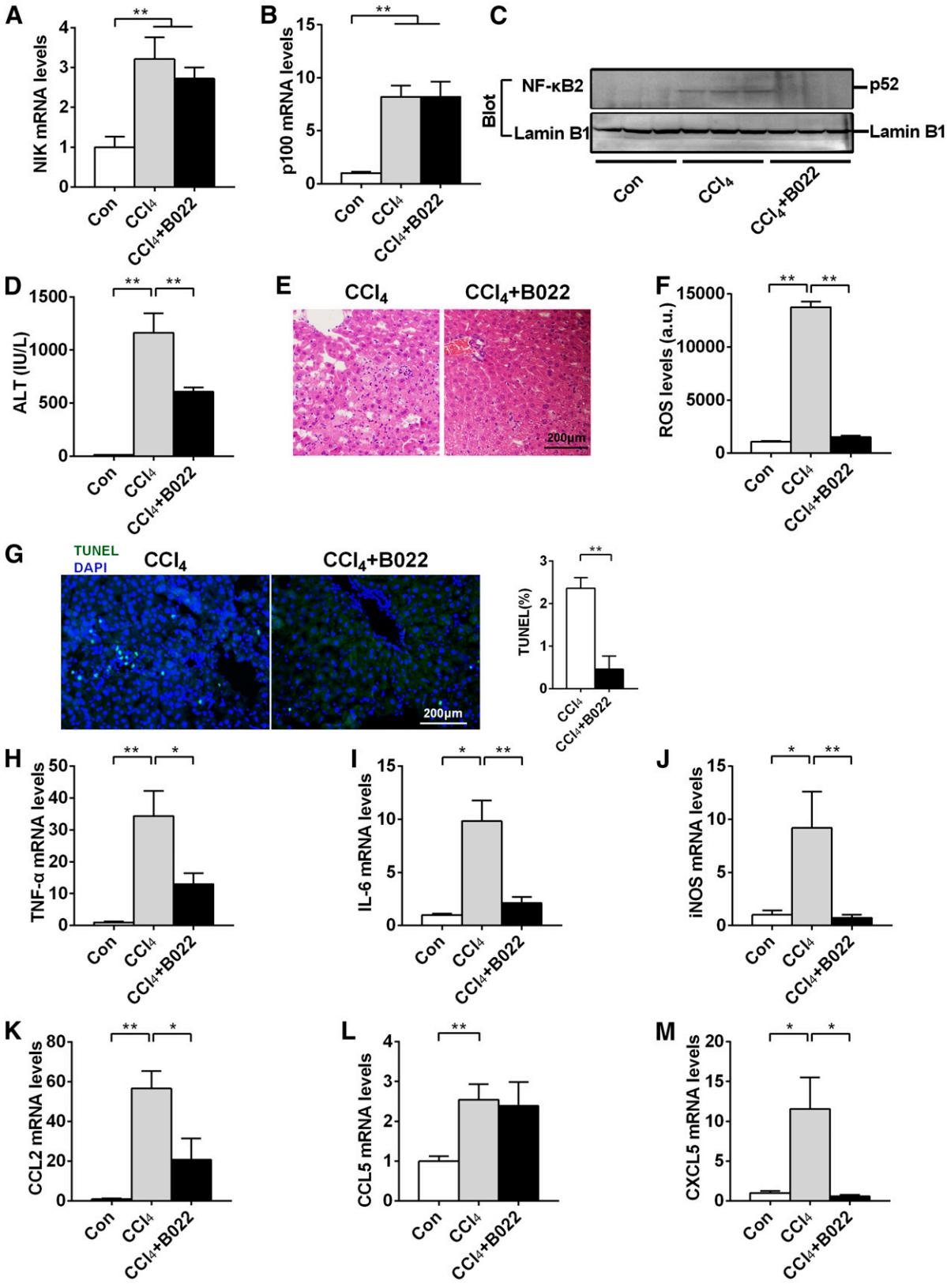

Figure 5. B022 ameliorates CCl_4-induced acute liver injury. For a CCl_4-induced liver injury mouse model, WT C57BL/6 mice were intraperitoneally injected with CCl_4 (2.5 ml/kg bodyweight, 10% in olive oil) and then with B022 (25 mg/kg bodyweight) or an equal volume of vehicle, *via* the tail vein at 0, 3, 6, or 9 h after CCl_4 injection. The mice were euthanized at 12 h after CCl_4 injection. *A*, *B*) Liver *NIK* (*A*) and *P100* (*B*) mRNA levels were measured by qPCR and normalized to 36B4 levels. *C*) Liver nucleus extracts were prepared and immunoblotted with antibodies to NF-κB2 or lamin B1. *D*–*G*) Blood ALT activity levels (*D*), H&E staining (*E*), liver reactive oxygen species (ROS) levels (*F*), and TUNEL staining of frozen liver sections (*G*) were observed and recorded. *H*–*M*) The expression of TNF-α (*H*), IL-6 (*I*), iNOS (*J*), CCL2 (*K*), CCL5 (*L*), and CXCL5 (*M*) was measured by qPCR and normalized to 36B4 levels. $n = 3$–9. *$P < 0.05$; **$P < 0.01$.

CCl_4-induced liver injury and inflammation (rather than 1 or 2 doses). Therefore, further modifications to B022 are needed to achieve better *in vivo* pharmacokinetic properties.

In summary, in our study, B022, an inhibitor of NIK, ameliorated NIK-induced liver inflammation, both *in vitro* and *in vivo*. B022 protected mice from NIK-induced liver failure and subsequent death. Moreover, it reduced CCl_4-induced liver inflammation and injury in mice. Our data suggest that further efforts toward the development of B022 analogs could yield therapeutic drugs for the treatment of liver inflammation, oxidative stress, and injury. FJ

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AUTHOR CONTRIBUTIONS

Z. Chen conceived of and designed the project, analyzed the data, and wrote the manuscript; X. Ren, X. Li., L. Jia, and H. Hou analyzed the data; L. Rui provided *STOP-NIK* mice and reagents and reviewed the manuscript; and D. Chen and Y. Zhao synthesized B022 and reviewed and edited the manuscript.

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