Pakistan J. Zool., vol. 52(5), pp 1863-1869, 2020 DOI: https://dx.doi.org/10.17582/journal.pjz/20191122091114

Induction of Nuclear Factor κB in Different First-Line Anti-tubercular Drug-induced Liver Injuries in Mice

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Article Information
Received 25 June 2019
Revised 30 July 2019
Accepted 04 March 2020
Available online 18 May 2020

Authors' Contribution
YL, DW and FF designed the study, performed experimental work and analyzed the data. XW, MZ, HZ, and ZS helped in microscopic examinations. YL and DW wrote the article.

Key words
Antituberculosis drug-induced liver injury, HL7702, CYP1A1, DNA methylation, 5-aza-2'-deoxycytidine

ABSTRACT

Nuclear factor κB (NF-κB) regulates the gene expression of eukaryotic cells. This study aims to reveal the changes in NF-κB during the development and progression of liver injury induced by anti-tuberculosis drugs in mice. A total of 168 Kunming mice were randomly divided into four experimental groups and one baseline group. The drug groups were orally administered with isoniazid (H), rifampicin (R), pyrazinamide (Z), and combination of these three drugs (HRZ) at 3, 5, 7, 10, and 15 days, respectively. Liver tissue pathologies and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities changed, indicating the occurrence of liver injury. Compared with the baseline group, the content of malondialdehyde (MDA) in each drug group gradually increased, and the activity of superoxide dismutase (SOD) gradually decreased, suggesting that oxidative stress reaction occurred in all drug groups. Compared with the baseline group, the mRNA and protein levels of NF-κB and TNF-α in the H group showed an increasing trend, and p-NF-κB and p-IκB also showed an increasing trend. However, these indicators increased first and then slightly decreased in the R, Z and HRZ groups. Among them, the significant changes in the indicators of the HRZ group were earlier than those of other drug groups and the changes were most obvious. The mRNA and protein of IκB in each drug group showed a gradual decline. The NF-κB pathway is activated in different mouse models of first-line anti-tuberculosis drug-induced liver injury and the activation level is different in each drug group. Among them, the NF-κB activation level of the combination therapy group is the highest, and the damage to hepatocyte is more serious than the single drug.

INTRODUCTION

First-line anti-tuberculosis drugs are widely used to curb the spread of tuberculosis, however the incidence of side effects is also increasing. Adverse effects of anti-tuberculosis drugs can affect multiple tissues and organs, and anti-tuberculosis drug-induced liver injury (ADLI) is the most severe one.

Even though many researches focused on ADLI, the mechanism of ADLI remains unclear because of its complexity. There are not doubts that ADLI is a multi-factor-mediated disease, in which oxidative stress is one of the most important aspects (Li *et al*., 2015). Large amounts of reactive oxygen species (ROS) are produced during anti-tuberculosis treatment; the combination of ROS with a liver enzyme or receptor on the cell membrane could trigger cell metabolism disorders and even death (Gupta *et al*., 2010). Nuclear factor (NF)-κB is the first eukaryotic transcription factor proven to be activated by ROS and is sensitive to oxidative stress; ROS and NF-κB demonstrate a positive feedback effect by activating each other (Morgan and Liu, 2011). NF-κB regulates gene expression in various eukaryotic cells as a transcription factor (Bakkar and Guttridge, 2010). NF-κB and its inhibitory protein IκB (NF-κB Inhibitor, IκB) are combined to be a trimmer in the stationary state, which covers the NF-κB nuclear localization sequence (nuclear translocation signal, NLS) and inhibits translocation. It makes NF-κB exists in the cytoplasm stably as an inactive form which does not participate in gene transcription (Liu *et al*., 2015). However, activation of NF-κB is essential for cellular survival as the response to oxidative stress (Choi *et al*., 2016).

As an important inflammatory cytokine, TNF-α can activate NF-κB which then promotes TNF-α expression inversely, therefore a vicious cycle emerges in the cytokine

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0030-9923/2020/0005-1863 $ 9.00/0

network (Liu *et al*., 2019). The NF-κB activity in liver Kupffer cells, hepatocytes, and sinusoidal endothelial cells is significantly increased in ethanol-caused liver damage which exhibits a high positive correlation with the extent of liver damage (Wang *et al*., 2017). Although NF-κB has been detected in various liver diseases, the relationship between ADLI and NF-κB still warrants further investigation. Tuberculosis chemotherapy mainly consists of a combination of isoniazid (INH), rifampicin (RFP), and pyrazinamide (PZA), it indicated that the interactions among these drugs increase hepatotoxicity (Miglani *et al*., 2016). However, the mechanisms of the liver toxicity which involves these three drugs and their combination are still unclear. In this study, mice were modeled with three first-line anti-TB drugs alone and in combination, the degrees of oxidation of liver tissue damage (malondialdehyde, MDA; superoxide dismutase, SOD) were assessed, and the mRNA and protein expression levels of NF-κB, IκB, and TNF-α in liver tissue were measured. Overall results showed that NF-κB is indeed involved in some processes of ADLI.

MATERIALS AND METHODS

Animals and modeling

There are 168 Kunming mice (SPF grade) in total, each weighing 18-22 g, half male and half female, which were obtained from China Fukang Biotechnology Limited (animal license number: SCXK (Beijing) 2016-0006). All animals were fed in SPF Laboratory Animal Center of North China University of Science and Technology, with an interior temperature of 18-22°C and a humidity of 50%-60%. All experiments were performed in accordance with the guidelines for animal research established by the Local Ethics Committee of Animal Experiments at North China University of Science and Technology (Certificate number: LX2018137)

Animal-free food and water were purchased from China Fukang Biotechnology Company. After adaptive feeding, all animals were randomly divided into four experimental groups and one baseline group. The experimental groups included groups H (INH 90 mg/Kg•d), R (RFP 135 mg/Kg•d), Z (PZA 315 mg/Kg•d), and HRZ (INH 90 mg/Kg•d + RFP 135 mg/Kg•d + PZA 315 mg/Kg•d) where the dose was converted from adult clinical one. And then each experimental group was randomly divided into five groups, namely, 3 d, 5d, 7 d, 10 d, and 15 d group, which corresponded to the mice sacrificed after gavage at 3, 5, 7, 10, and 15 days (n=8 in each group). And other eight rats of baseline group were sacrificed before starting gavage.

Histopathological changes

Mice were sacrificed by cervical dislocation. Liver tissues were isolated, fixed in 10% formalin, sectioned after embedding in paraffin, and then stained with hematoxylin-eosin (HE).

Detection of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels

The blood samples of each group were collected by removing mouse' eyeballs after the last administration with 12 h fasting. Blood samples were centrifuged at 3000 r/min for 10 min to separate serum, which was stored in the refrigerator at -80°C. Serum ALT and AST were detected with a HITACHI 7600-020 automatic biochemical analyzer.

Measurement of MDA content and SOD vitality of liver tissue

A portion of liver tissue was sectioned and then added with normal saline by nine times of the volume to prepare 10% homogenate. The homogenate was centrifuged at 3000 r/min for 10 min, and then the supernatant was collected. MDA content and SOD activity were measured using corresponding kits (Nanjing Jiancheng Bioengineering Institute).

Analyses of liver NF-κB, IκB and TNF-α mRNA expression levels

Total RNA was extracted from liver tissue with Trizol method and then transcribed reversely into cDNA using M-MLV first-strand synthesis system kit. SYBR Green real-time quantitative PCR amplification was performed to detect the mRNA expression of NF-κB, IκB and TNF-α. The amplification conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 30 s; and 72°C for 5 min.

Analysis of liver NF-κB, p-NF-κB, IκB, p-IκB and TNF-α proteins expression

The tissue homogenate was combined with the amount of liver tissue and saline respectively 1:4, centrifuged at 3000 r/min for 10 min, and then the supernatant was collected. The NF-κB, p-NF-κB, IκB, p-IκB and TNF-α proteins were detected by enzyme-linked immunosorbent assay kit (Beijing Dong Song Bo Industry Biotechnology Limited Offer).

Statistical analysis

Data were processed using SPSS 22.0. Distributed measurement data were described by mean ± standard deviation. Mean values were compared using Brown-Forsythe of ANOVA, and then the comparison between two groups was performed using LSD method. Differences were considered statistically significant at $P<0.05$.

RESULTS

Anti-tuberculosis drugs prompted histopathologic damages of liver

The hepatocytes of the baseline group were arranged radially around the central vein of the liver, and the cells were clear and compact in arrangement without apparent morphological changes. However, each drug group showed obvious morphological changes at the beginning of 3rd day, wherein the cells swelled around the central vein, cytoplasmic vacuolization and nuclear condensation, and the cell gap enlarged or infiltrated by inflammatory cells respectively. On the 15th day, obvious hepatocyte damage was observed in each drug group, suggesting that the model of liver injury induced by anti-tuberculosis drugs has been successfully established. In addition, hepatocyte damage in the HRZ group was found to be more severe than in the single drug groups (Fig. 1).

Anti-tuberculosis drugs increased serum ALT and AST levels

Compared with the baseline group, the levels of ALT and AST in the H, R, and Z groups gradually increased with the time of administration (all $P<0.05$), whereas

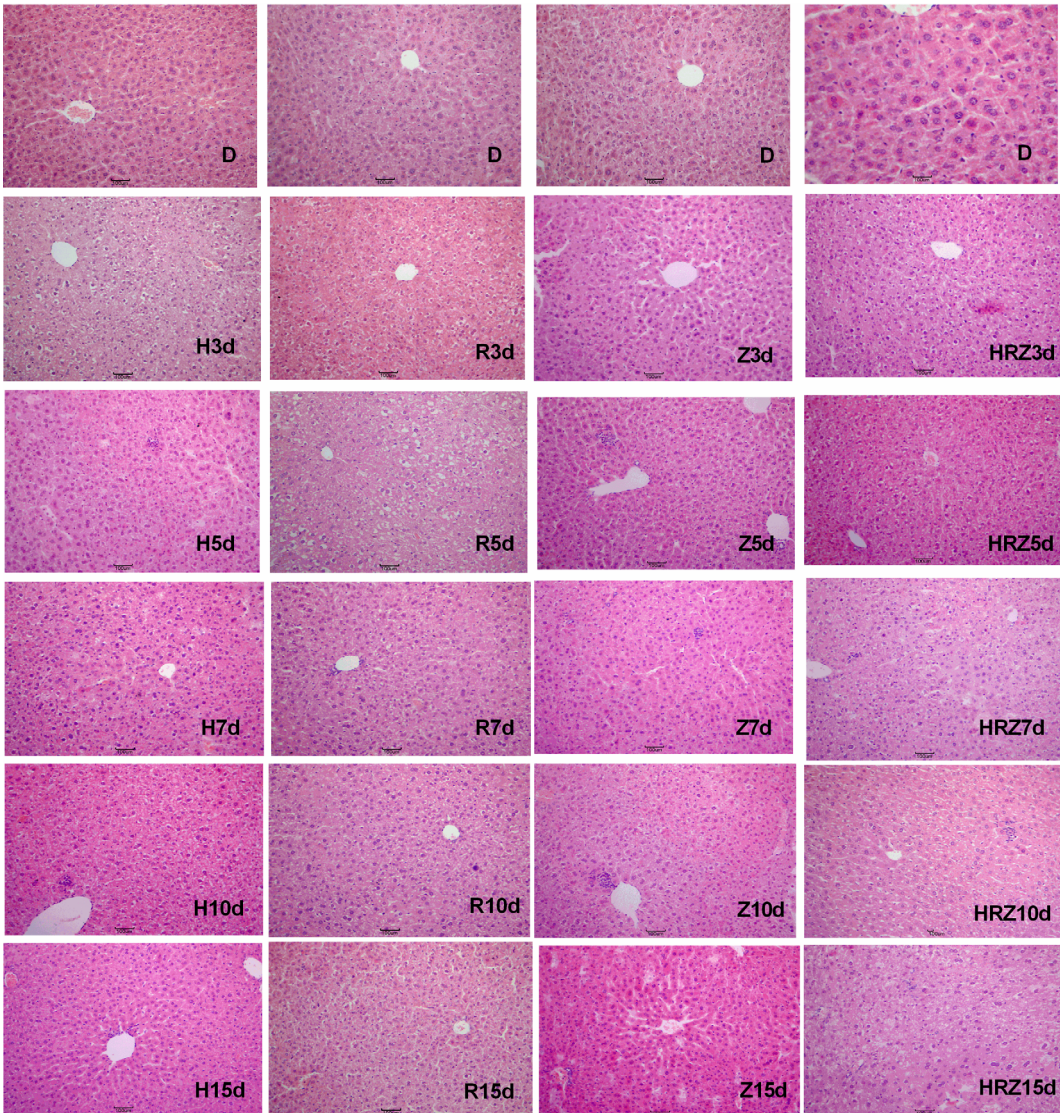

Fig. 1. Histopathological changes in the liver tissue of mice in each group. Comments: Mice were treated with vehicle for control or anti-tuberculosis drugs for 3, 5, 7, 10, and 15 days, and then liver samples were processed routinely and stained with H&E for morphologic evaluation (original magnification × 100). D: Control; H: isoniazid, R: rifampicin, Z: pyrazinamide, and HRZ: INH + RFP + PZA.

those in HRZ group initially increased and then decreased (all P<0.05). In the HRZ group, ALT and AST indicators showed significant changes on the 5th day, which were earlier than other drug groups (all P<0.05) (Fig. 2A, 2B).

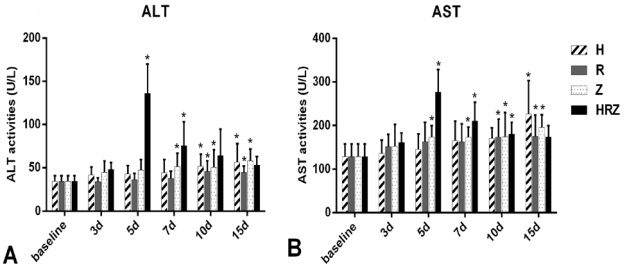

Fig. 2. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of the different groups. Data are expressed as mean ± SD of n = 8 animals per group. *P<0.05.

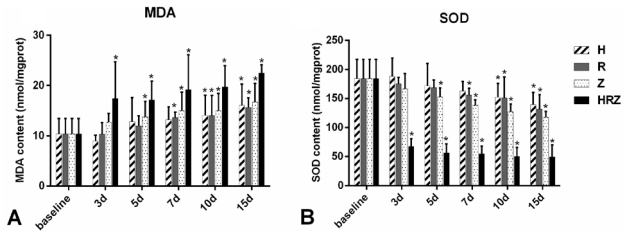

Fig. 3. MDA content and SOD activity in different groups. Data are expressed as mean ± SD of n = 8 animals per group. *P<0.05.

Anti-tuberculosis drugs increased MDA level but decreased SOD level

Compared with the baseline group, the levels of MDA in groups H, R, Z, and HRZ gradually increased during prolonged treatment period (all P<0.05), while the levels of SOD changed in the opposite way (all P<0.05). Compared with the drug groups, the changes in MDA and SOD in the HRZ group were most significant (all P<0.05) (Fig. 3A, 3B).

Expression trends of NF-κB, IκB and TNF-α mRNA and protein under the anti-tuberculosis drugs

Compared with the baseline group, the mRNA, protein and phosphorylated protein levels of NF-κB in H group gradually increased from 1st to 15th day (all P<0.05), whereas those in R, Z, and HRZ groups initially increased and then decreased (all P<0.05). Moreover, the mRNA and protein of the HRZ group showed significant changes on the 5th day, and the p-NF-κB showed significant changes on the 3th day, which was all earlier than other drug groups. Compared with the drug groups, the changes of the indicators in the HRZ group were the most obvious (all P<0.05) (Fig. 4A, 4C).

Compared with the baseline group, the mRNA and protein levels of IκB in each drug group gradually decreased from 1st to 15th day (all P<0.05), and both showed significant changes on the 7th day (all P<0.05). Compared with the drug groups, the degree of decline was most obvious in the H group and the HRZ group. The p-IκB showed an increasing trend in the H group, while the R, Z and HRZ groups showed increase and then a slight downward trend. The p-IκB in the HRZ group showed a significant change on the 3rd day, which was all earlier than other drug groups. Compared with the drug groups, the HRZ group showed the most obvious change in each index (all P<0.05) (Fig. 4D, 4F).

The mRNA and protein expression levels of TNF-α were similar to those of NF-κB. Compared with the baseline group, the mRNA and protein expression levels of TNF-α in the H group gradually increased from 1st to 15th day (all P<0.05), while the R, Z and HRZ groups increased first and then decreased (all P<0.05). Compared with the drug groups, the changes of the indicators in the HRZ group were the most obvious (all P<0.05) (Fig. 4G, 4H).

DISCUSSION

The MDA and SOD are a group of important oxidases and anti-oxidant enzymes in the body (Jiang *et al*., 2019). In this study, MDA and SOD of each drug group showed significant changes, and MDA and SOD showed opposite trends, indicating that both single drug and combination drug will damage liver cells through lipid peroxidation. When the body's oxidation and anti-oxidant balance are destroyed, a large amount of oxygen free radicals are generated, which reduces SOD activity (Sies *et al*., 2017). In addition, we found that the degree of oxidative damage in the HRZ group was more serious, which may be due to the combination of the three drugs to produce more oxygen free radicals, resulting in a significant reduction in SOD activity.

Under normal physiological conditions, NF-κB binds to its inhibitory protein IκB in the cytoplasm, leaving NF-κB in the cytoplasm in an inactive state (Rao *et al*., 2010). When cells were stimulated by oxidative stress, IκB may degrade and separate from NF-κB (Zuo *et al*., 2017). NF-κB is then activated to shift into the nucleus. Activated NF-κB binds to the κB site of the promoter region of the inflammatory mediator's target gene, resulting in overexpression of inflammatory mediator genes (Lian *et al*., 2019). In the results of this study, the mRNA and protein of NF-κB and IκB, as well as p-NF-κB and p-IκB, were significantly changed in each drug group, suggesting that the NF-κB pathway was activated.

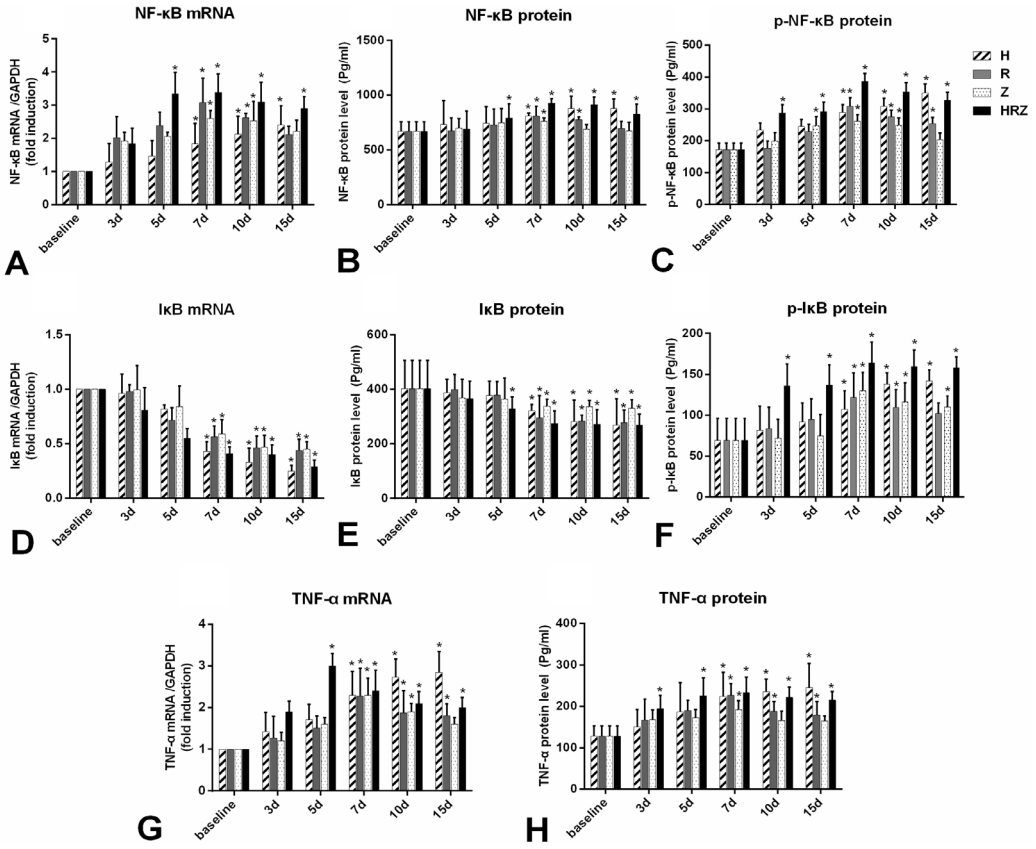

Fig. 4. The mRNA and protein expression levels of NF-kB, IkB and TNF-α in different groups. Data are expressed as mean ± SD of n = 8 animals per group. *P<0.05.

We found that the expression level of NF-κB in group H showed an increasing trend. However, the expression levels of NF-κB in the R, Z and HRZ groups increased first and then decreased slightly, and the difference in the HRZ group was the most significant. Study has shown that immunomodulatory effects of RFP can inhibit NF-κB activation and reduce the production of pro-inflammatory factors (Wang *et al*., 2013; Zhu *et al*., 2017). At the same time, human mononuclear cells and mice treated with PZA can inhibit the activation of NF-κB by regulating the NF-κB-dependent pathway (Manca *et al*., 2013; Sakala *et al*., 2013). Therefore, we can speculate that INH induces liver damage by inducing oxidative stress and then activating the NF-κB pathway. RFP and PZA can cause hepatotoxicity and induce oxidative stress to induce NF-κB activation, but at the same time may also play an immunomodulatory role to inhibit NF-κB activation, explaining the reasons for the increase in R, Z and HRZ groups.

Cytokines induce nuclear translocation of NF-κB by IκB serine phosphorylation (Chen *et al*., 2017). When NF-κB binds to the κB locus, transcription of certain genes begins or increases (Kastl *et al*., 2014). In fact, TNF-α is even involved in the subacute phase of liver injury (Filliol *et al*., 2017). Without IκB, NF-κB is more exposed to TNF-α in mouse fibroblasts, which leads to over-activation of NF-κB (Liu *et al*., 2019). At the same time, other studies have shown that the increase of inflammatory cytokines (TNF-α and IL-6) can also lead to the activation of NF-κB (Chen *et al*., 2008; Liang *et al*., 2017). In the present study, the mRNA and protein expression of TNF-α in H group increased gradually and were higher than those of the other three groups from 10 days. This result indicates that TNF-α is increased when the body takes the INH drug. When NF-κB is activated, it also promotes TNF-α gene transcription, which in turn activates NF-κB. However, the TNF-α index in the R, Z and HRZ groups increased first and then decreased with the prolongation of the administration time. We speculate that the cause may be related to the immunomodulatory effects exerted by RFP and PZA. In addition, study has shown that RFP has the potential to inhibit TNF-induced NF-κB activation while reducing TNF-α production (Pahlevan *et al*., 2002). The

decrease in proinflammatory cytokines and chemokine levels may also occur after PZA treatment (Pahlevan *et al*., 2002). These observations are consistent with our findings, but the specific mechanisms need to be further explored.

In summary, our results suggested that NF-κB pathway was activitied in different first-line anti-tubercular drug-induced liver injuries in mice. However, the NF-κB signaling pathway network is enormous and precise, and its role in ADLI deserves further study. Despite the rapid development of modern medicine, few reliable drugs may protect the liver from damage and/or promote the regeneration of liver cells. Therefore, the mechanism by which the activated NF-κB signaling pathway is terminated may become another research focus in the future.

CONCLUSIONS

The NF-κB pathway is activated in different mouse models of first-line anti-tuberculosis drug-induced liver injury and the activation level is different in each drug group. Among them, the NF-κB activation level of the combination therapy group is the highest, and the damage to hepatocyte is more serious than the single drug.

ACKNOWLEDGEMENT

This work was supported by the Natural Science Foundation of Hebei Province, China (No: H2016209300).

Statement of conflict of interest

The authors have declared no conflicts of interest.

REFERENCES

Bakkar, N. and Guttridge, D.C., 2010. Nf-kappab signaling: A tale of two pathways in skeletal myogenesis. *Physiol. Rev.,* **90**: 495-511. https://doi.org/10.1152/physrev.00040.2009

Chen, S., Maini, R., Bai, X., Nangreave, R.C., Dedkova, L.M. and Hecht, S.M., 2017. Incorporation of phosphorylated tyrosine into proteins: In vitro translation and study of phosphorylated ikappab-alpha and its interaction with nf-kappab. *J. Am. chem. Soc.,* **139**: 14098-14108. https://doi.org/10.1021/jacs.7b05168

Chen, T.M., Subeq, Y.M., Lee, R.P., Chiou, T.W. and Hsu, B.G., 2008. Single dose intravenous thioacetamide administration as a model of acute liver damage in rats. *Int. J. exp. Pathol.,* **89**: 223-231. https://doi.org/10.1111/j.1365-2613.2008.00576.x

Choi, M.H., Ow, J.R., Yang, N.D. and Taneja, R., 2016. Oxidative stress-mediated skeletal muscle degeneration: Molecules, mechanisms, and therapies. *Oxid. med. Cell. Longev.,* **2016**: 6842568. https://doi.org/10.1155/2016/6842568

Filliol, A., Piquet-Pellorce, C., Raguenes-Nicol, C., Dion, S., Farooq, M., Lucas-Clerc, C., Vandenabeele, P., Bertrand, M.J.M., Le Seyec, J. and Samson, M., 2017. Ripk1 protects hepatocytes from kupffer cells-mediated tnf-induced apoptosis in mouse models of pamp-induced hepatitis. *J. Hepatol.*, **66**: 1205-1213. https://doi.org/10.1016/j.jhep.2017.01.005

Gupta, S.C., Sundaram, C., Reuter, S. and Aggarwal, B.B., 2010. Inhibiting nf-kappab activation by small molecules as a therapeutic strategy. *Biochim. biophys. Acta,* **1799**: 775-787. https://doi.org/10.1016/j.bbagrm.2010.05.004

Jiang, Z., Guo, X., Zhang, K., Sekaran, G., Cao, B., Zhao, Q., Zhang, S., Kirby, G.M. and Zhang, X., 2019. The essential oils and eucalyptol from artemisia vulgaris l. Prevent acetaminophen-induced liver injury by activating nrf2-keap1 and enhancing apap clearance through non-toxic metabolic pathway. *Front. Pharmacol.,* **10**: 782. https://doi.org/10.3389/fphar.2019.00782

Kastl, L., Sauer, S.W., Ruppert, T., Beissbarth, T., Becker, M.S., Suss, D., Krammer, P.H. and Gulow, K., 2014. Tnf-alpha mediates mitochondrial uncoupling and enhances ros-dependent cell migration via nf-kappab activation in liver cells. *FEBS Lett.,* **588**: 175-183. https://doi.org/10.1016/j.febslet.2013.11.033

Li, S., Tan, H.Y., Wang, N., Zhang, Z.J., Lao, L., Wong, C.W. and Feng, Y., 2015. The role of oxidative stress and antioxidants in liver diseases. *Int. J. mol. Sci,* **16**: 26087-26124. https://doi.org/10.3390/ijms161125942

Lian, Y.H., Fang, J., Zhou, H.D., Jiang, H.F. and Xie, K.J., 2019. Sufentanil preconditioning protects against hepatic ischemia-reperfusion injury by suppressing inflammation. *Med. Sci. Monit,* **25**: 2265-2273. https://doi.org/10.12659/MSM.913145

Liang, S., Chen, Z., Jiang, G., Zhou, Y., Liu, Q., Su, Q., Wei, W., Du, J. and Wang, H., 2017. Activation of gper suppresses migration and angiogenesis of triple negative breast cancer via inhibition of nf-kappab/il-6 signals. *Cancer Lett.,* **386**: 12-23. https://doi.org/10.1016/j.canlet.2016.11.003

Liu, B., Sun, L., Liu, Q., Gong, C., Yao, Y., Lv, X., Lin, L., Yao, H., Su, F., Li, D., Zeng, M. and Song, E., 2015. A cytoplasmic nf-kappab interacting long noncoding rna blocks ikappab phosphorylation and suppresses breast cancer metastasis. *Cancer*

Cell, **27**: 370-381. https://doi.org/10.1016/j.ccell.2015.02.004

Liu, H., Wu, X., Luo, J., Wang, X., Guo, H., Feng, D., Zhao, L., Bai, H., Song, M., Liu, X., Guo, W., Li, X., Yue, L., Wang, B. and Qu, Y., 2019. Pterostilbene attenuates astrocytic inflammation and neuronal oxidative injury after ischemia-reperfusion by inhibiting nf-kappab phosphorylation. *Front. Immunol.,* **10**: 2408. https://doi.org/10.3389/fimmu.2019.02408

Manca, C., Koo, M.S., Peixoto, B., Fallows, D., Kaplan, G. and Subbian, S., 2013. Host targeted activity of pyrazinamide in mycobacterium tuberculosis infection. *PLoS One,* **8**: e74082. https://doi.org/10.1371/journal.pone.0074082

Miglani, S., Patyar, R.R., Patyar, S. and Reshi, M.R., 2016. Effect of goat milk on hepatotoxicity induced by antitubercular drugs in rats. *J. Fd. Drug. Anal.,* **24**: 716-721. https://doi.org/10.1016/j.jfda.2016.03.012

Morgan, M.J. and Liu, Z.G., 2011. Crosstalk of reactive oxygen species and nf-kappab signaling. *Cell. Res.,* **21**: 103-115. https://doi.org/10.1038/cr.2010.178

Pahlevan, A.A., Wright, D.J., Bradley, L., Smith, C. and Foxwell, B.M., 2002. Potential of rifamides to inhibit tnf-induced nf-kappab activation. *J. Antimicrob. Chemother.,* **49**: 531-534. https://doi.org/10.1093/jac/49.3.531

Rao, P., Hayden, M.S., Long, M., Scott, M.L., West, A.P., Zhang, D., Oeckinghaus, A., Lynch, C., Hoffmann, A., Baltimore, D. and Ghosh, S., 2010. Ikappabbeta acts to inhibit and activate gene expression during the inflammatory response. *Nature,* **466**: 1115-1119. https://doi.org/10.1038/nature09283

Sakala, I.G., Eickhoff, C.S., Blazevic, A., Moheno, P., Silver, R.F. and Hoft, D.F., 2013. Dipterinyl calcium pentahydrate inhibits intracellular mycobacterial growth in human monocytes via the c-c chemokine mip-1beta and nitric oxide. *Infect. Immun.,* **81**: 1974-1983. https://doi.org/10.1128/IAI.01393-12

Sies, H., Berndt, C. and Jones, D.P., 2017. Oxidative stress. *Annu. Rev. Biochem.,* **86**: 715-748. https://doi.org/10.1146/annurev-biochem-061516-045037

Wang, M., Shen, G., Xu L., Liu, X., Brown, J.M., Feng, D., Ross, R.A., Gao, B., Liangpunsakul, S. and Ju, C., 2017. Il-1 receptor like 1 protects against alcoholic liver injury by limiting nf-kappab activation in hepatic macrophages. *J. Hepatol.,* **68**: 109-117. https://doi.org/10.1016/j.jhep.2017.08.023

Wang, X., Grace, P.M., Pham, M.N., Cheng, K., Strand, K.A., Smith, C., Li, J., Watkins, L.R. and Yin, H., 2013. Rifampin inhibits toll-like receptor 4 signaling by targeting myeloid differentiation protein 2 and attenuates neuropathic pain. *FASEB J.,* **27**: 2713-2722. https://doi.org/10.1096/fj.12-222992

Zhu, L., Kang, H., Guo, C.A., Fan, W.S., Wang, Y.M., Deng, L.F. and Yan, Z.Q., 2017. Rifampin suppresses osteoclastogenesis and titanium particle-induced osteolysis via modulating rankl signaling pathways. *Biochem. biophys. Res. Commun.,* **484**: 64-70. https://doi.org/10.1016/j.bbrc.2017.01.071

Zuo, T., Zhu, M., Xu, W., Wang, Z. and Song, H., 2017. Iridoids with genipin stem nucleus inhibit lipopolysaccharide-induced inflammation and oxidative stress by blocking the nf-kappab pathway in polycystic ovary syndrome. *Cell. Physiol. Biochem.,* **43**: 1855-1865. https://doi.org/10.1159/000484074