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SIRT1 alleviates isoniazid-induced hepatocyte injury by reducing histone acetylation in the IL-6 promoter region

Yiyang Zhang, Yingshu Li, Jinfeng Li, Biao Li, Yingzhi Chong, Guoying Zheng, Shufeng Sun, Fumin Feng*

School of Public Health, North China University of Science and Technology, No. 21 Bohai Road, Caofeidian Eco-city, Tangshan, Hebei 063210, PR China

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ABSTRACT

Silent information regulator 1 (SIRT1) is a type III histone deacetylase that is related to the inhibition of the inflammatory response. The aim of this study was to investigate the regulation of SIRT1 on isoniazid-induced hepatocyte injury and the possible mechanism of histone modification. We found that compared with the blank control group, expression of SIRT1 was decreased in the isoniazid group and that expression of NF-κB p65 was increased, leading to an increase of the expression of inflammatory cytokines Interleukin-6 (IL-6) and Tumour necrosis factor alpha (TNF-α). The level of histone H3K9 acetylation in the promoter region of IL-6 was increased as well. Addition of a SIRT1 agonist (SRT1720) alleviated the inflammatory reaction caused by isoniazid, while the use of a SIRT1 inhibitor (EX527) aggravated the inflammatory damage to cells. In conclusion, these findings indicated that during the period of isoniazid-induced hepatocyte injury, SIRT1 levels were decreased and inflammatory factor levels were increased. Activation of SIRT1 may reduce hepatocyte injury by reducing the level of histone H3K9 acetylation in the promoter region of the IL-6 gene.

1. Introduction

Currently, tuberculosis remains a global public health problem. The World Health Organization (WHO) Global Tuberculosis Report 2017 indicated that in 2016, there were approximately 10.4 million new cases of tuberculosis worldwide, of which approximately 1.67 million resulted in death. Isoniazid (INH) is one of the major first-line anti-tuberculosis drugs recommended by WHO, and the most serious side effect in clinical research is anti-tuberculosis drug-induced liver injury (ADLI) [1]. At present, it is believed that the inflammatory reaction induced by INH drug metabolism plays an important role in the process of liver injury, and may become a major target for ADLI prevention and treatment [2].

During the development of ADLI, epigenetic factors have received increasing attention. Among them, histone modification is a key component of epigenetics, and histone acetylation is a significant part of histone modification. As the main component of chromatin, the lysine residues at the end of the amino acids often change the configuration of chromatin by covalent modification, which leads to gene transcription activation or gene silencing [3]. Histone deacetylase (HDAC) can remove the acetyl group on the terminal lysine residue of the histone amino acid terminus to restore their positive charge, thereby increasing the affinity between DNA and histone, making them more compact and inhibiting the transcription of genes [4]. Silent information regulator 1 (SIRT1) is a class of nicotinamide adenine dinucleotide (NAD+) dependent type III histone deacetylase that can regulate the equilibrium state of the biological clock and the aging of the body by deacetylating histone H3K9 and H4K16 in the promoter region of the clock gene Per2 to abate its transcription. [5]. After the specific knockout of SIRT1 in mouse cardiomyocytes, it was found that poly [adenosine diphosphate (ADP)- ribose] polymerase 1 (PARP1) was activated when the cardiomyocyte was stimulated, and SIRT1 could restrain the activation of the PARP1 gene promoter or the direct deacetylation of PARP1 to protect cardiomyocytes from PARP1-mediated death [6]. A number of studies have demonstrated that SIRT1 is involved in multiple functional activities of cell senescence, antioxidative stress and inhibition of inflammation [7–16].

Interleukin-6 (IL-6) is an important inflammatory cytokine, and its gene promoter region contains important transcriptional regulatory elements regulated by proteins such as Nuclear factor-κB (NF-κB) and Activator protein-1 (AP-1) [17]. The normal expression of IL-6 contributes to the steady state of immune homeostasis, while the excessive production can lead to a series of inflammatory damage, such as compensatory and decompensated alcoholic cirrhosis, Crohn's disease and

* Corresponding author.
E-mail address: fm_feng@sina.com (F. Feng).

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Table 1
Primer sequences.

Gene	Primer	Sequence (5′-3′)
Human GAPDH	F	GAAGGTCGGAGTCAACGGATT
	R	CCTGGAAGATGGTGATGGGAT
Human SIRT1	F	CATAGACACGCTGGAACAGG
	R	TTGAGGGAAGACCCAATAACA
Human NF-κB p65	F	GCGAGAGGAGCACAGATACC
	R	GCACAGCATTCAGGTCGTAG
Human IL-6	F	CACACAGACAGCCACTCACC
	R	GCTCTGGCTTGTTCCTCACT
Human TNF-α	F	CAGCCTCTTCTCCTTCCTGA
	R	TGAGGTACAGGCCCTCTGAT

glomerulonephritis [18–20]. Tumour necrosis factor alpha (TNF-α) is another important inflammatory factor in the immune system of the body that can mediate many biological effects. The levels of IL-6 and TNF-α in the serum of patients with chronic hepatitis B increase gradually with the prolongation of the condition. IL-6 and TNF-α are involved in the pathogenesis of hepatitis B [21]. In view of this, the expression of inflammatory factors is closely related to the occurrence of liver diseases. It is essential to effectively intervene in the disorder of the inflammatory environment in the body and restore the normal expression of the inflammatory mediators. Recently, the abnormal modification of DNA and histone is often associated with gene expression, and the epigenetic modification mechanism of inflammatory gene expression has attracted more and more attention. Existing studies have noted that IL-6 plays an important role in the development of pulmonary fibrosis induced by paraquat, whose promoter region can be regulated by histone acetylation. The application of HDAC inhibitors can enhance the level of histone H3K9 acetylation in the promoter region of IL-6 and further aggravate the occurrence of pulmonary fibrosis [22]. As a target molecule of SIRT1, NF-κB p65 can regulate the expression of downstream inflammatory factors IL-6 and TNF-α. Therefore, we speculate that in the process of hepatocyte injury induced by isoniazid, the increase of pro-inflammatory response mediated by inflammatory regulators and even the level of histone H3K9 acetylation in the promoter region of inflammatory factor IL-6 may be related to the expression of histone deacetylase SIRT1.

In this study, isoniazid was used to stimulate human normal liver cells to observe the changes in the expression of SIRT1, the occurrence of inflammatory reaction and the possible histone modification mechanism during ADLI. To further observe the effect of SIRTI on inflammatory factors and to explore the regulatory role of SIRT1 in inflammatory factors in ADLI, specific SIRT1 agonists and inhibitors were added to show the influence on the expression of SIRT1, especially using IL-6 as an example. This will provide the basis for ADLI prevention and effective liver protection treatment for tuberculosis patients.

2. Materials and methods

2.1. Materials

The human normal liver cell line HL-7702 was obtained from the Chinese Academy of Sciences (Shanghai, China). Isoniazid was purchased from TCI (Tokyo, Japan). SIRT1 agonist SRT1720 and inhibitor EX527 were obtained from Selleck (USA). PrimeScript™ RT Master Mix Kit and SYBR® Premix Ex Taq™ II PCR Kit were purchased from Takara Biotechnology (Dalian, China). Alanine aminotransferase assay kit and aspartate aminotransferase assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Rabbit anti-SIRT1 antibody was purchased from EPITOMICS (USA). Rabbit anti-NF-κB p65 antibody and goat anti-rabbit IgG (HRP) were purchased from Abcam (USA). Human IL-6 ELISA Kit and Human TNF-a ELISA Kit were obtained from DG (Beijing, China). CHIP-Kit was purchased from ABclonal (Wuhan, China).

2.2. Cell culture and treatment

The cells were grown in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% foetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin in a cell incubator with 5% CO_2 at 37 °C. After 24 h of pre-culture, the medium was changed and treated accordingly. Cells and supernatants were harvested after another 48 h of culture.

In order to determine the optimal combination concentration of SIRT1 agonist SRT1720 and inhibitor EX527, we detect the cell viability of HL-7702 cells cultured for 48 h under different concentration gradients. The results showed that the agonist concentration of 1 μmol/L and the inhibitor concentration of 1 μmol/L were their maximum non-toxic doses respectively. The experiment was conducted in the following treatment groups: blank control group (1640 medium), INH group (1640 medium containing 800 μg/mL isoniazid), INH + SRT1720 group (1640 medium containing 800 μg/mL isoniazid and 1 μmol/L SRT1720), SRT1720 group (1640 medium containing 1 μmol/L SRT1720), INH + EX527 group (1640 medium containing

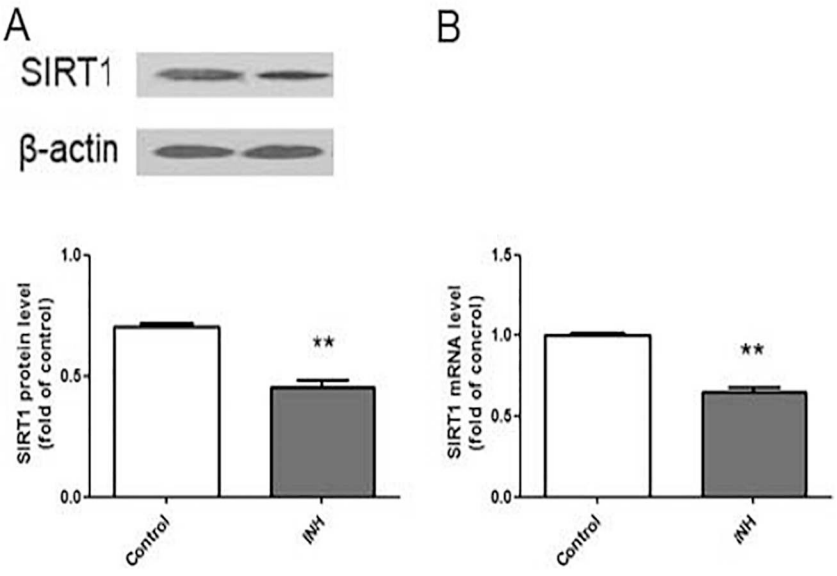

Fig. 1. Changes of protein (A) and mRNA (B) expression of SIRT1 in the control group and INH group. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h and then SIRT1 protein expression was determined by Western blotting and mRNA expression was determined by real-time PCR. Data indicate the means of six independent experiments ± SD. ** $P < 0.01$ vs control group.

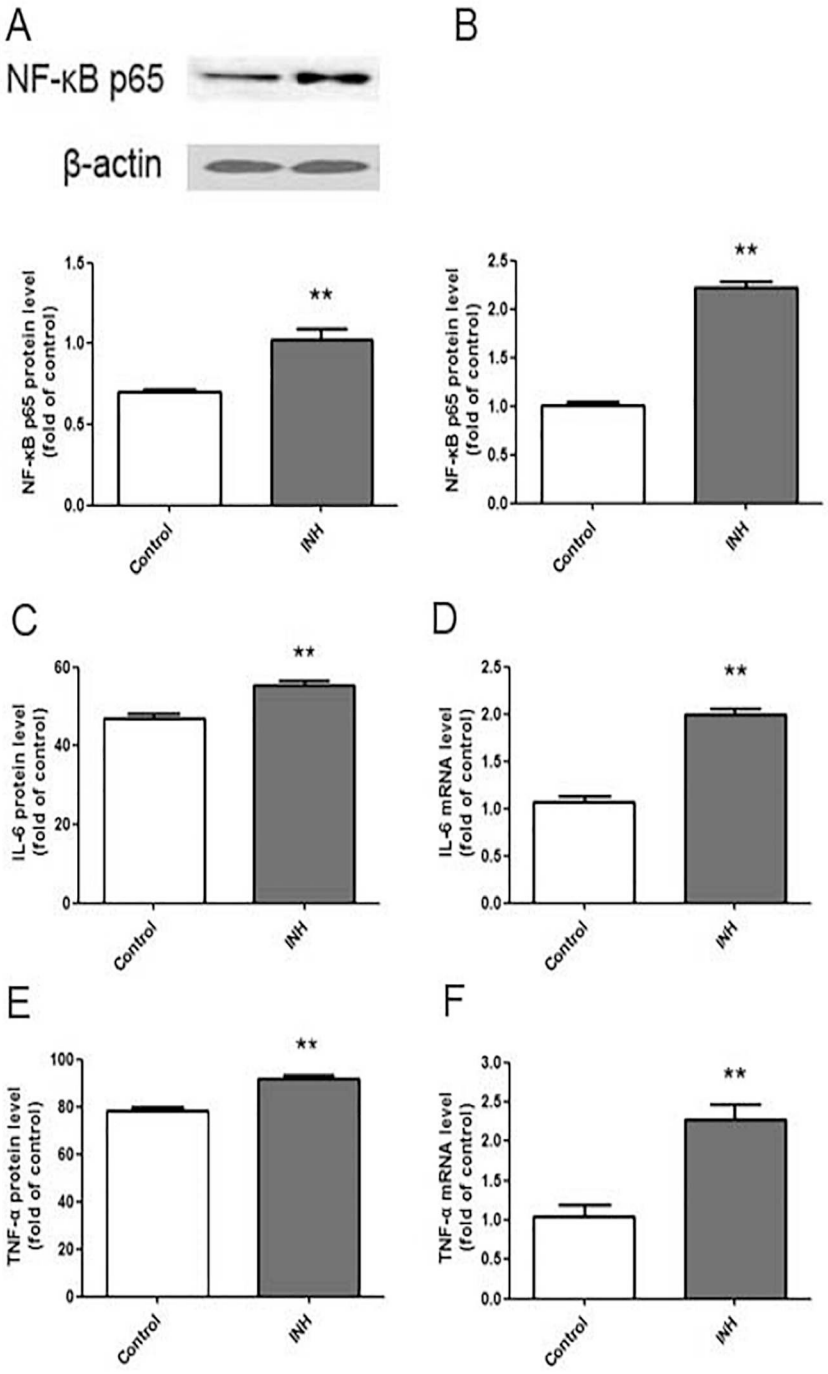

Fig. 2. Changes in the protein (A, C and E) and mRNA (B, D and F) expression of NF-kB p65, IL-6 and TNF-a in the control group and INH group. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h and then the NF-κB p65 expression was determined by Western blotting, the IL-6 and TNF-a expression was determined by ELISA, and the mRNA expression of NF-κB p65, IL-6 and TNF-a was determined by real-time PCR. Data indicate the means of six independent experiments ± SD. ** $P < 0.01$ vs control group.

800 μg/mL isoniazid and 1 μmol/L EX527) and EX527 group (1640 medium containing 1 μmol/L EX527).

2.3. Cell morphology assay

The cells were stained with haematoxylin-eosin, and their morphological changes were observed under a light microscope.

2.4. Real-time PCR

Total RNA was extracted from cells using TRIzol and cDNAs were synthesized from total RNA using PrimeScript RT Master Mix following the manufacturer's instructions. Real-time PCR was performed with the SYBR Premix Ex Taq II. The relative gene expression was quantitatively analysed by the comparative Ct method ($2^{-\Delta\Delta ct}$), using GAPDH level as control. Primer pair sequences are shown in Table 1.

2.5. Reitman-Frankel and ELISA

The supernatant of the cell culture was collected and the commercial ALT, AST and ELISA test kit instructions were strictly followed to determine the level of each indicator.

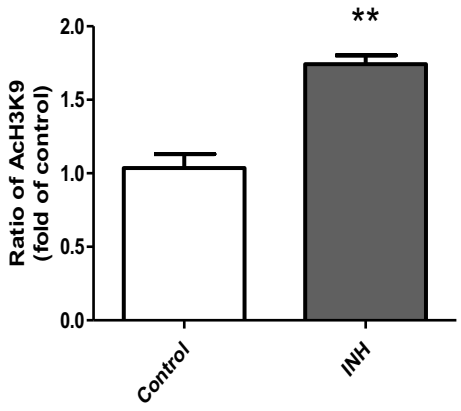

Fig. 3. Ratio of acetylated H3K9 in the promoter region of IL-6 in the control group and INH group. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h and then the acetylation of histone H3K9 in the promoter region of IL-6 was determined by Chip-PCR. Data indicate the means of six independent experiments ± SD. ** $P < 0.01$ vs control group.

2.6. Western blotting

The proteins were extracted with RIPA lysis buffer containing protease inhibitor cocktail. Protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat dry milk and then incubated overnight at 4 °C with a rabbit anti-SIRT1 (1:2000), anti-NF-κB p65 (1:50000), or anti-β-actin (1:2000) antibody. A goat anti-rabbit IgG-HRP antibody (1:2000) and the enhanced chemiluminescent substrate were used for detection.

2.7. Chromatin immunoprecipitation analysis

The operations were completed strictly according to the instructions of the CHIP-kit. The main steps are as follows: 1) protein-DNA cross-linking and lysis of cells; 2) ultrasonic fragmentation of chromatin; 3) co-immunoprecipitation; 4) de-crosslinking of protein-DNA complexes; and 5) real-time PCR analysis of the level of histone H3K9 acetylation in the promoter region of IL-6. The IL-6 gene structure was analysed using the UCSC database at http://genome.ucsc.edu. The IL-6 promoter region gene primers were as follows: the upstream primer sequence was 5′-GTAGAGCTCATCTCTCCCACAAG-3′, and the downstream primer sequence was 5′-ACTCCTGATAGTGGCCCTCG-3′.

2.8. Statistical analysis

The results are presented as the means ± standard deviation. Statistical analyses between two groups were performed by unpaired Student's *t*-test. Differences among groups were tested by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Changes of related indicators under the effect of isoniazid

3.1.1. Changes of ALT and AST activity in cell supernatants after isoniazid treatment

Compared with the blank control group (4.95 ± 0.25 U/L), isoniazid induction increased the ALT activity of the supernatant (10.45 ± 0.62 U/L) ($P < 0.01$). The AST activity (12.89 ± 0.54 U/L) was also significantly higher than in the control group (7.58 ± 0.52 U/L) ($P < 0.01$). The above results suggest that the ADLI cell model was successfully constructed.

3.1.2. Changes of SIRT1 protein and mRNA expression in isoniazid-induced cells

The expression of SIRT1 protein (Fig. 1A) and mRNA (Fig. 1B) in cells stimulated by isoniazid was reduced, and there was a significant difference compared with the blank control group ($P < 0.01$), suggesting that the change of SIRT1 expression was involved in the process of hepatocyte injury induced by isoniazid.

3.1.3. Changes of NF-κB p65, IL-6 and TNF-a protein and mRNA expression in isoniazid-induced cells

Isoniazid stimulation increased the protein and mRNA expression of NF-κB p65 (Fig. 2A–B), IL-6 (Fig. 2C–D) and TNF-α (Fig. 2E–F) in comparison with that in the blank control group ($P < 0.01$), suggesting that the cells in the INH group had inflammatory damage.

3.1.4. Changes of histone H3K9 acetylation level in the IL-6 gene promoter induced by isoniazid

The acetylation level of histone H3K9 in the promoter region of the IL-6 gene in the INH group was examined. Compared with the blank control group, the level of histone H3K9 acetylation in the IL-6 gene promoter was elevated in the INH group ($P < 0.01$), indicating that the histone modification mechanism is involved in the process of isoniazid-induced hepatocyte injury (Fig. 3).

3.2. The effect of SIRT1 expression change on inflammatory injury of cells

3.2.1. Effects of SIRT1 agonist and inhibitor on SIRT1 protein and mRNA expression

The combination of isoniazid and SRT1720 increased the expression of SIRT1 protein (Fig. 4A) and mRNA (Fig. 4B), which was significantly different from that of the INH group ($P < 0.05$), while the combined

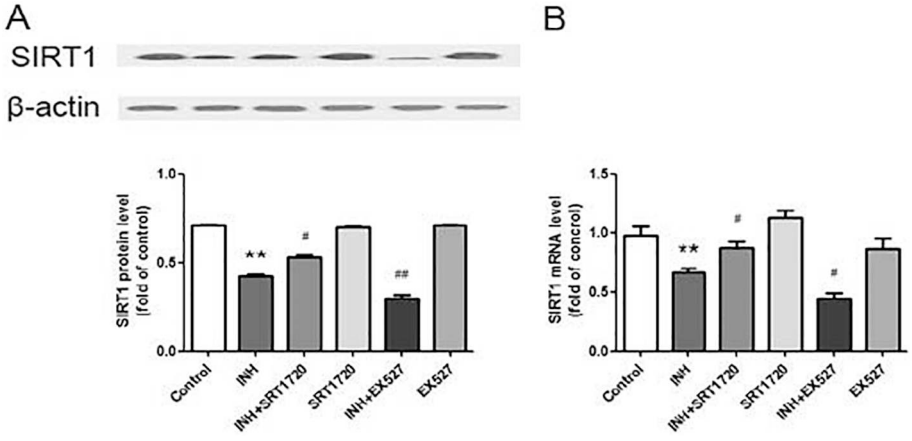

Fig. 4. Changes in the protein (A) and mRNA (B) expression of SIRT1 in different groups. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h, the cells of the INH + SRT1720 group and INH + EX527 group were stimulated separately with INH (800 μg/mL) + SRT1720 (1 μmol/L) and INH (800 μg/mL) + EX527 (1 μmol/L) for 48 h, and the cells of the SRT1720 group and EX527 group were stimulated separately with SRT1720 (1 μmol/L) and EX527 (1 μmol/L) for 48 h; then, the SIRT1 protein expression was determined by Western blotting and the mRNA expression was determined by real-time PCR. Data indicate the means of six independent experiments ± SD. ** $P < 0.01$ vs control group. # $P < 0.05$ vs INH group. ## $P < 0.01$ vs INH group.

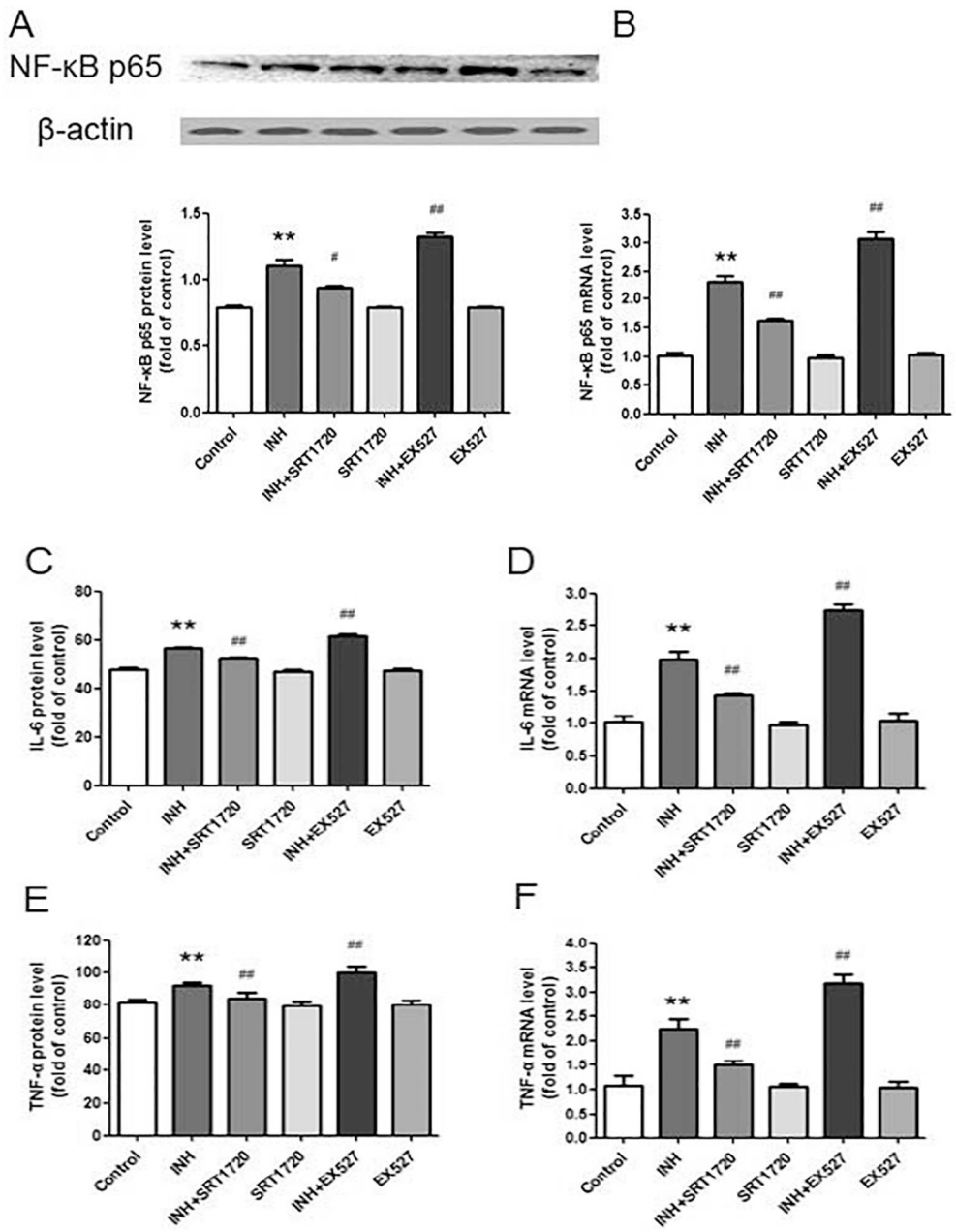

Fig. 5. Changes in the protein (A, C and E) and mRNA (B, D and F) expression of NF-kB p65, IL-6 and TNF-a in different groups. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h, the cells of the INH + SRT1720 group and INH + EX527 group were stimulated separately with INH (800 μg/mL) + SRT1720 (1 μmol/L) and INH (800 μg/mL) + EX527 (1 μmol/L) for 48 h, and the cells of the SRT1720 group and EX527 group were stimulated separately with SRT1720 (1 μmol/L) and EX527 (1 μmol/L) for 48 h. Then, NF-κB p65 expression was determined by Western blotting, IL-6 and TNF-a expression was determined by ELISA, and the mRNA expression of NF-κB p65, IL-6 and TNF-a was determined by real-time PCR. Data indicate the means of six independent experiments ± SD. ** $P < 0.01$ vs control group. $^{\#}$ $P < 0.05$ vs INH group. $^{\#\#}$ $P < 0.01$ vs INH group.

use of EX527 further reduced the expression of SIRT1 protein and mRNA ($P < 0.05$). This finding suggests that SIRT1 agonist can activate SIRT1 expression, while SIRT1 inhibitor inhibits SIRT1 expression.

3.2.2. Effects of SIRT1 expression changes on NF-κB p65, IL-6 and TNF-α protein and mRNA expression

The combination of isoniazid and SRT1720 can inhibit the increase of NF-κB p65 (Fig. 5A–B), IL-6 (Fig. 5C–D) and TNF-α (Fig. 5E–F) protein and mRNA expression caused by isoniazid stimulation, which showed a significant difference from the INH group ($P < 0.05$). This finding suggests that the activation of SIRT1 can reduce the expression of inflammatory regulators, thereby reducing the expression of inflammatory factors and thus reducing the inflammatory response induced by isoniazid. The combination with EX527 further increased the expression of NF-κB p65 (Fig. 5A–B), IL-6 (Fig. 5C–D) and TNF-α (Fig. 5E–F) protein and mRNA, which showed a significant difference from the INH group ($P < 0.01$). This suggests that the inhibition of SIRT1 may increase the expression of inflammatory regulators, which in turn increases the expression of inflammatory cytokines and aggravates the occurrence of inflammatory lesions. There was no significant difference in the expression of inflammatory factors between the SRT1720 group, the EX527 group and the blank control group, suggesting that the SIRT1 agonist and inhibitor had no toxic side effects on cells.

3.2.3. Effects of SIRT1 expression changes on histone H3K9 acetylation level in the IL-6 gene promoter region

The effect of SIRT1 on histone acetylation in the promoter region of the IL-6 gene was further analysed. The results showed that the level of histone H3K9 acetylation in the promoter region of the IL-6 gene was decreased after combined use of SRT1720, and the combined use of EX527 increased the acetylation level, which was significantly different from that of the INH group ($P < 0.01$) (Fig. 6.). This finding suggested that SIRT1 may play a role in the deacetylation of histone H3K9 in the promoter region of the IL-6 gene to reduce inflammation caused by isoniazid.

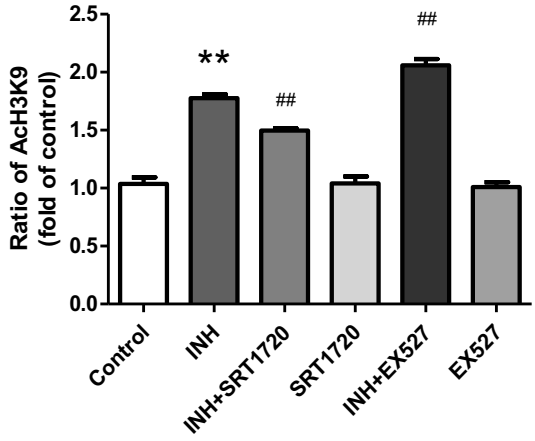

Fig. 6. Ratio of acetylated H3K9 in the promoter region of IL-6 in different groups. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h, the cells of the INH + SRT1720 group and INH + EX527 group were stimulated separately with INH (800 μg/mL) + SRT1720 (1 μmol/L) and INH (800 μg/mL) + EX527 (1 μmol/L) for 48 h, and the cells of the SRT1720 group and EX527 group were stimulated separately with SRT1720 (1 μmol/L) and EX527 (1 μmol/L) for 48 h. Then, the acetylation of histone H3K9 in the promoter region of IL-6 was determined by Chip-PCR. Data indicate the means of six independent experiments ± SD. $^{**}P < 0.01$ vs control group. $^{\#\#}P < 0.01$ vs INH group.

3.3. Morphological changes of cells in each group

The morphological changes of each group were consistent with histone modification. That is, the cells of the INH group had apoptosis damage, and the cytoplasm was loose and the staining became weak. The combined use of SRT1720 reduced the damage to liver cells, while the combined use of EX527 aggravated the cell damage. In the blank control group, the SRT1720 group and the EX527 group, the liver cells were dense and full, indicating that the cells in the control group were normal and the agonists and inhibitors had no side effects (Fig. 7).

4. Discussion

In this study, by using isoniazid to stimulate normal human hepatocyte HL-7702 cells that was cultured for 48 h to establish the ADLI cell model, it was found that SIRT1 expression was reduced in the INH group, indicating that the change of SIRT1 expression may be associated with ADLI. In recent years, epigenetics has played an important role in the development of liver diseases. Epigenetics includes DNA methylation, histone modification, chromatin remodelling and non-coding RNA regulation [23]. Histone acetylation is an important component of histone modification, which is regulated by histone acetyltransferase (HAT) and histone deacetylase (HDAC). Therefore, the expression of histone acetylation is often accompanied by changes in the expression level of HAT or HDAC. SIRT1 is a type III histone deacetylase and is one of its main organs of expression [24]. The expression levels of SIRT1 drop in liver fibrosis mice, liver cirrhosis and activated hepatic stellate cells, while compared with the wild-type mice, the degree of liver fibrosis in SIRT1 knockout mice is aggravated by carbon tetrachloride [25]. The use of salvianolic acid B to upregulate the expression of SIRT1 can increase the level of deacetylation of p53, which in turn raises the activity of antioxidant enzymes, leading to a reduction in the degree of ethanol-induced acute liver injury [26]. Other Studies have shown that resveratrol can reduce the occurrence of acute liver injury in mice induced by isoniazid and rifampicin by regulating the expression of SIRT1 [27]. In vivo studies, the down-regulation of SIRT1/FXR pathway may be a key link in the process of liver injury induced by isoniazid in rats [28]. All these observations suggested that the SIRT1 expression change is closely related to the occurrence of liver disease. The results of this study can also provide evidence for this. SIRT1 is associated with multiple functional activities, such as cell senescence, anti-oxidative stress, inhibition of inflammation and mitochondrial homeostasis [5–14]. In peripheral blood mononuclear cells, *N*-acetyltransferase 2 is the target of SIRT1, and the degree of acetylation of NAT2 by SIRT1 may have potential effects on different physiological processes, toxicity or tumorigenesis after exposure to drugs or carcinogens [29]. From this point of view, changes in the expression of SIRT1 are likely to affect the development of related diseases. To observe the occurrence of inflammatory reactions during the development of ADLI caused by isoniazid, we detected increased expression of NF-κB

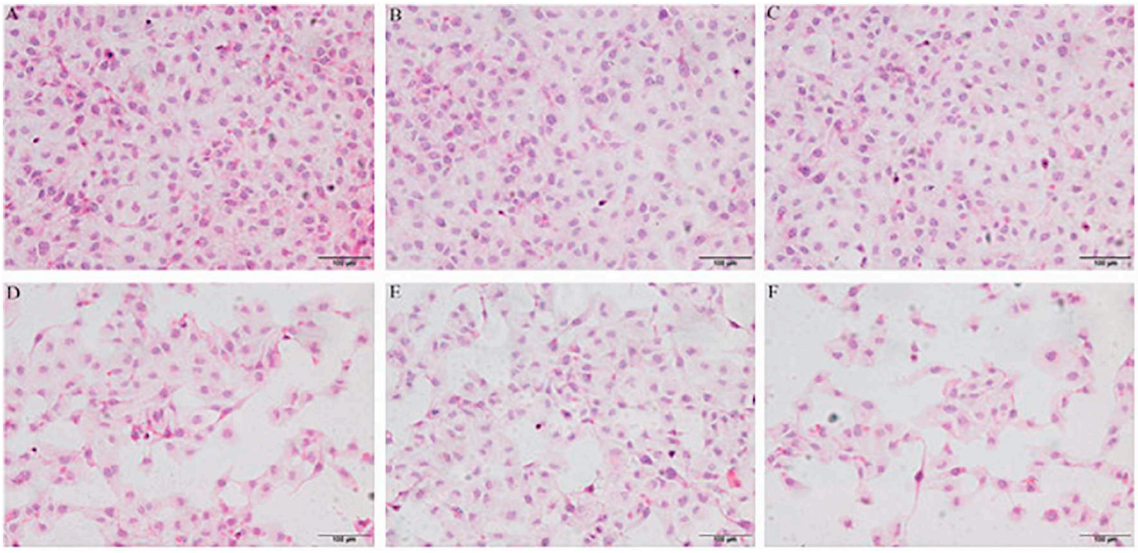

Fig. 7. Morphological changes of HL-7702 in different groups (HE, × 100). A means control group, B means SRT1720 group, C means EX527 group, D means INH group, E means INH + SRT1720 group, F means INH + EX527 group. The cells of the INH group were stimulated with INH (800 μg/mL) for 48 h, the cells of the INH + SRT1720 group and INH + EX527 group were stimulated separately with INH (800 μg/mL) + SRT1720 (1 μmol/L) and INH (800 μg/mL) + EX527 (1 μmol/L) for 48 h, and the cells of SRT1720 group and EX527 group were stimulated separately with SRT1720 (1 μmol/L) and EX527 (1 μmol/L) for 48 h. Then the morphological changes were determined by H&E staining.

p65 and inflammatory cytokines IL-6 and TNF-α in the INH group. The acetylation level of histone H3K9 was elevated in the promoter region of IL-6 gene. These results suggest that the occurrence of inflammatory reaction during isoniazid-induced hepatocyte injury may be related to changes in SIRT1 expression, and histone modification mechanisms may be involved.

Studies have confirmed that SIRT1 can regulate the expression of inflammatory factors and then participate in the inflammatory immune system of the body to regulate the occurrence and development of tumours [30–37]. The mechanism underlying SIRT1's inhibition of inflammatory responses is not currently clear, but most people believe that it is related to the suppression of inflammatory signalling pathways. Among these, the most-studied one is the inhibitory effect of SIRT1 on the NF-κB signalling pathway. Earlier studies have noted that in the inflammatory response, the p65 subunit of NF-κB is a direct target of SIRT1. SIRT1 reduces the level of the acetylation of NF-κB p65 by deacetylation of its 310th lysine residues, thereby inhibiting transcription of downstream inflammatory factors [38]. To further investigate the physiological functions that are involved in the differential expression of SIRT1, SIRT1 expression was altered by adding a specific SIRT1 agonist and inhibitor to observe changes in the downstream expression of inflammatory cytokines. The results showed that after isoniazid combined with SRT1720, the expression of NF-κB p65 was decreased, as was the expression of downstream IL-6 and TNF-α. The expression of NF-κB p65, IL-6 and TNF-α was further increased with the combination of EX527, indicating that the expression changes affected the inflammatory reaction caused by isoniazid. This result is probably due to the deacetylation of NF-κB p65 by SIRT1. Some scholars believe that HDAC can inhibit the expression of inflammatory factors in lung and BMDM cells, so its agonists may have an important clinical value in asthma and cystic fibrosis [39]. As a histone deacetylase, SIRT1 not only has effects on the deacetylation of non-histone proteins but also plays a more important role in the regulation of histone deacetylation. Based on the previous regulatory effect on histone acetylation of related genes in the promoter region by SIRT1 [14,15,20], we examined the influence of SIRT1 expression change on histone H3K9 acetylation levels in the inflammatory cytokine IL-6 promoter region. The experimental results showed that the level of histone H3K9 acetylation in the promoter region of IL-6 was decreased after combined use of SRT1720, and the combined use of EX527 further increased the acetylation level. The above findings suggested that SIRT1 may also deacetylate histone H3K9 of the inflammatory factor IL-6 gene promoter and reduce IL-6 expression to reduce the inflammatory damage caused by isoniazid.

In this study, we hypothesized that low expression of SIRT1 and high expression of inflammatory factors participate in the process of isoniazid-induced normal human hepatocyte injury. However, only a human normal hepatocyte line HL-7702 has been used, and the diversity of cell lines was not taken into consideration. The idea that the involvement of SIRT1 in ADLI may be through the deacetylation of histone H3K9 in the inflammatory cytokine IL-6 promoter region is also only validated using the isoniazid drug, which does not confirm the universality of the mechanism and still needs to be verified and compared in other human normal hepatocyte lines and with other types of anti-tuberculosis drugs. Deeper studies should be conducted to clarify the relevant mechanisms by both in vivo, in vitro studies and clinical experiments, and lay a more detailed theoretical foundation for future preventive treatment of ADLI.

In summary, our results provide direct evidence that SIRT1 inhibits the expression of isoniazid-induced inflammatory cytokines IL-6 and TNF-α in human normal hepatocytes. In addition, the inhibitory effect of SIRT1 on inflammation may be achieved by deacetylation of histone H3K9 in the promoter region of the IL-6 gene. These findings may provide some basis for the prevention of ADLI and effective liver protection treatment for tuberculosis patients.

Conflict of interest

The authors state no conflict of interest.

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