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Original Article

Effect of N-acetylcysteine on apoptosis and autophagy of macrophages infected with *Mycobacterium tuberculosis*

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Abstract

Introduction: The purpose of this study was to observe the effect of N-acetylcysteine (NAC) on oxidative stress (OS), intracellular *Mycobacterium tuberculosis* (MTB) load, apoptosis, and autophagy of macrophages infected with H37Rv MTB. In addition, we explored the possible mechanism of action, to provide a rationale for the use of NAC in the treatment of tuberculosis.

Methodology: We divided THP-1 macrophages into four groups: control, control + NAC, H37Rv, and H37Rv + NAC. OS, apoptosis, autophagy and intracellular MTB colony-forming unit (CFU) indexes were measured at 0, 4, 24, and 48 hours, respectively. Then, various indicator changes were systematically compared.

Results: The levels of reactive oxygen species (ROS), malondialdehyde (MDA), apoptosis rate, and LC3II/ β-actin ratio in the H37Rv group increased at 4 hours and reached their peak at 48 hours. The ROS and MDA in the H37Rv + NAC group were lower than those in the H37Rv group. CFU in the H37Rv + NAC group increased at 24 hours and decreased at 48 hours after treatment with NAC, relative to the H37Rv group. In addition, the H37Rv + NAC group showed a decrease in LC3II/β-actin ratio 48 hours after NAC treatment, compared to the H37Rv group.

Conclusions: MTB infection can lead to an increase in macrophage OS, apoptosis, and autophagy levels. However, after treatment with NAC, the growth of MTB in macrophages is inhibited, and OS and autophagy levels are reduced. The antioxidant effect and inhibitory effect of NAC on MTB are related to MTB-mediated macrophage OS and autophagy.

Key words: *Mycobacterium tuberculosis*; N-acetylcysteine; macrophages; oxidative stress; apoptosis; autophagy.

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Introduction

There were 10.6 million new cases of tuberculosis in the world in 2022, and among all the new cases, there were 410,000 (3.9%) cases of multidrug/rifampicin resistant tuberculosis. The global death toll from tuberculosis was 1.3 million. The success rate of anti-tuberculosis treatment has improved compared to 2021. The success rate of drug sensitive tuberculosis patients was 88%, and that of multi-drug resistant/drug-resistant tuberculosis patients rose to 63% [1].

Oxidative stress (OS) refers to a state in the body where there is an imbalance between oxidation and antioxidant activity, with a tendency towards oxidation. The ratio of reactive oxygen species (ROS) to antioxidants may be inextricably linked to the occurrence and progression of diseases, particularly respiratory tract diseases [2,3]. According to recent research, after infection with *Mycobacterium tuberculosis* (MTB), macrophages produce a large amount of ROS [4]. MTB may be killed or inhibited by ROS, but it also generates many mechanisms to evade killing [5]. Studies have shown that combining anti-tuberculosis drugs with adjuvants may limit the ability of MTB to counteract host OS response [6].

Macrophages, as phagocytes of the innate immune system, have a pivotal role in maintaining tissue homeostasis and responding to pathogens. Through apoptosis and autophagy, macrophages influence tuberculosis development [7]. The manipulation of host cell death pathways by MTB is complex. It can be divided into at least two stages: early anti-apoptotic stage and late pro-necrotic stage [8]. MTB activates the exogenous apoptosis pathway of macrophages, and classical apoptosis promotes the host defense of tuberculosis [9]. Research has shown that xenophagy, a typical form of autophagy, can effectively combat MTB infection in the early stages of infection [10]. But there are also studies showing that autophagy prevents early proinflammatory responses and neutrophil recruitment during MTB infection without affecting the pathogen burden in macrophages [11].

N-acetylcysteine (NAC) is an amino acid derived from the N-acetylation of L-cysteine. Studies have demonstrated that NAC can play an antioxidant role and it has been used to treat many diseases [12], including lung diseases [13] such as chronic obstructive pulmonary disease (COPD) [14] and bronchial asthma [15]. Mohanty *et al.* found that NAC may inhibit the cytokine storm after coronavirus disease 2019 (COVID-19) infection, thereby reducing the lesion and enhancing the prognosis [16]. Concerning tuberculosis (TB), research has shown that NAC has a certain anti-TB effect [17].

However, there are few reports on whether NAC affects the apoptosis and autophagy of macrophages infected with MTB [18]. Additionally, there is a scarcity of studies reporting whether the antioxidant effect of NAC and the reduction of intracellular colony-forming unit (CFU) in macrophages infected with MTB are related to host apoptosis and autophagy.

The purpose of this study was to observe the effect of NAC on OS, intracellular MTB load, apoptosis and autophagy of macrophages infected with MTB; and explore its possible relationship. It provides the premise for the future exploration of mechanisms and the basis for the application of NAC in the treatment of tuberculosis.

Methodology

Bacteria

The H37Rv MTB (American Type Culture Collection, ATCC, VA, USA) were grown in Middlebrook 7H9 broth (BD, New Jersey, USA) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) and 0.05% tween-80 (Sigma, Saint Louis, USA), or on Middlebrook 7H10 agar (BD, New Jersey, USA) supplemented with 10% OADC. After 10–20 days of culture, the OD600 absorbance of the bacterial solution reached 0.6–1.0 (about 1–3 × 10^8 CFU/mL). This experiment used only H37Rv MTB, and did not use clinical or drug-resistant strains.

Cell cultures

The human monocyte-like cell line THP-1 was obtained from ATCC (VA, USA) and differentiated into mature macrophages after being treated with phorbol ester (PMA; Multi Sciences, Hangzhou, China). Briefly, THP-1 cells were cultured in Roswell Park Memorial Institute (RPMI) medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA) at 37°C in 5% CO_2. THP-1 cells were seeded on a 12-well plate or a 6-well plate for the experiments. Each well contained 10^6 and 2 × 10^6 cells, respectively. They were cultured for 36 hours in the presence of phorbol ester (PMA) (100 ng/mL) to induce macrophage differentiation. The cells were washed and incubated for an additional 24 hours in a fresh medium without PMA until their use in experiments.

Macrophage infection

THP-1 was seeded on a 12-well plate or a 6-well plate. MTB H37Rv strains were grown to the mid-logarithmic phase at OD600 = 0.6. MTB were collected and washed twice in 1 × phosphate buffered saline (PBS) buffer containing 0.05% tween-80 and were then pelleted and thoroughly resuspended using the cell culture medium with 0.05% tween-80. Macrophage cells were then infected with MTB to reach a multiplicity of infection (MOI = 10) for 3 hours at 37°C.

Mycobacterial quantification

The cells were washed thrice with PBS buffer after the end of MTB infection for bacterial CFU counting [19,20]. Bacterial numbers were determined by serial dilution of bacterial cultures and cell lysates in Middlebrook 7H10 medium (BD, New Jersey, USA) supplemented with 10% OADC. CFU numbers were determined after 3 weeks of incubation at 37°C.

Cytotoxicity test of NAC

We measured the cell viability of macrophages treated with NAC (0 mM, 2.5 mM, 5 mM, 10 mM, and 20 mM) using the Cell Count Kit 8 (CCK-8; Dojindo, Shanghai, China). Cells (5 × 10^4) were inoculated into a 96 well culture plate to induce differentiation, and incubated with different concentrations of NAC for 4, 8, 24, and 48 hours. After incubation, 10 μL CCK-8

reagent was added and kept for 4 hours to measure the OD_{450} value using a microplate reader (BioTek, Winooski, USA). Finally, cell viability was calculated.

Treatment of NAC and experimental grouping

Based on the cytotoxicity test of NAC, 10 mM was selected as the experimental concentration. The pH of the NAC containing medium was adjusted to be consistent with the untreated medium using 5N NaOH. We divided the macrophages into four groups which included control group (uninfected, untreated with NAC), control + NAC (uninfected, treated with NAC), H37Rv (infected with H37Rv MTB, untreated with NAC), and H37Rv + NAC (infected with H37Rv MTB, treated with NAC) groups. OS, apoptosis, autophagy and intracellular MTB CFU indexes were measured at 0, 4, 24 and 48 hours, respectively. All experiments were performed at least 3 times.

Measurement of intracellular ROS

Macrophages were isolated from the culture plate using trypsin (0.25%), washed, and resuspended in serum-free medium. Cells were stained at 37 °C and 5% CO_2 for 20 minutes using ROS Assay Kit (Beyotime, Shanghai, China), and then analyzed using flow cytometry. The differences in mean fluorescence intensity (MFI) between groups were compared.

Measurement of lipid peroxidation

Cellular extracts were prepared by sonication in ice-cold buffer. After sonication, lysed cells were centrifuged at 10,000 × g for 10 min to remove debris. The supernatant was subjected to measurement of malondialdehyde (MDA) levels and protein contents. We used a lipid peroxidation MDA assay kit (Solarbio, Beijing, China) to measure the change in lipid peroxidation. We used a BCA Protein Assay Kit (Tiangen, Beijing, China) to quantify protein concentration. MDA levels were then normalized to milligram protein. We used the same procedure to lyse the cells and determine the protein contents in the following assays unless otherwise indicated.

Measurement of antioxidant enzyme activity

We used the Catalase (CAT) kit (Solarbio, Beijing, China) for the determination of catalase. The cells were treated according to the method of MDA determination and protein quantification. The enzyme activities were tested according to the manufacturer's instructions. The OD value was measured with a BioTek Epoch microplate reader (BioTek, Winooski, USA).

Apoptosis analysis

Apoptosis was evaluated by Muse Annexin V and Dead Cell Kit (Luminex, Austin, TX, USA). The cells were previously detached from the culture plates using trypsin (0.25%), washed, and resuspended in phenol and serum-free medium. The apoptosis rate was calculated by flow cytometry using Guava (Luminex, Austin, TX, USA) and Flowjo software (Treestar, Ashland, OR, USA).

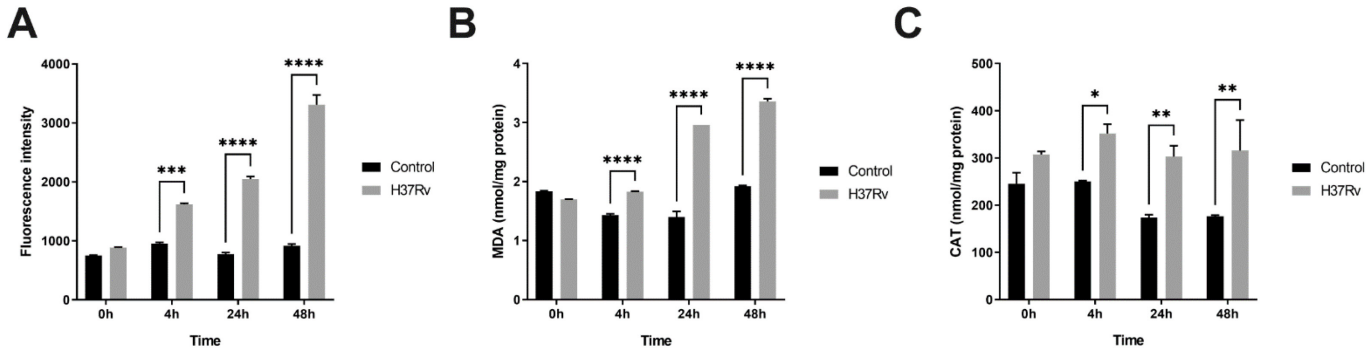
Autophagy analysis

The macrophages were lysed in 1 mM phenylmethanesulfonyl fluoride (PMSF) for immunoblot analysis. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Color Mixed Protein Marker (11–180 KD) (Solarbio, cat#PR1910, Beijing, China) was used for labeling protein molecular weights. The blots were blocked with 5% non-fat dry milk in tris-buffered saline with tween-20 (TBST) for 1 hour at room temperature, and subsequently incubated with primary antibodies overnight at 4 °C. LC3B antibody produced in rabbit (Sigma, cat# L7543; 1:1000 dilution, Saint Louis, USA), and SQSTM1/p62 Rabbit mAb (Abclonal, cat# A19700, 1:1000 dilution, Wuhan, China) were used. Monoclonal anti-β-actin antibodies produced in mouse (Sigma, cat#A2228, 1:5000 dilution, Saint Louis, USA) were used as primary antibodies. Subsequently, the membranes were incubated with goat anti-mouse IgG or goat anti-rabbit IgG (Zhongshan Golden Bridge Biotechnology, Beijing, China) conjugated to horseradish peroxidase (HRP) at a dilution of 1:10,000 in blocking buffer for 1 hour at room temperature. Finally, the blots were developed by Immobilon Western Chemiluminescent HRP Substrate (Solarbio, Beijing, China) and exposed to Amersham ImageQuant 800 (Cytiva, Washington, DC, USA). Densitometric quantification of the protein bands was performed using the ImageJ software (Treestar, Ashland, OR, USA).

Statistical analysis

The data shown in graphs were presented as mean ± standard error of the mean (SEM). The number of experimental replicates were indicated in the figure legend. Statistical analyses were performed with GraphPad Prism 9.0 software (GraphPad Software, Boston, Massachusetts, USA) using either unpaired two-tailed t-test for comparison between two groups or one-way analysis of variance (ANOVA). Statistical

Figure 1. The effect of MTB H37Rv infection on oxidative stress.



A. Reactive oxygen species (ROS) levels; **B.** Malondialdehyde (MDA) levels; **C.** Catalase (CAT) levels. Control group, uninfected; H37Rv group, infected with H37Rv MTB. MTB, Mycobacterium tuberculosis; H37Rv, infected with H37Rv MTB. Values are presented as the mean ± SEM (n = 3). *, p < 0.05; ***, p < 0.001; ****, p<0.0001.

differences were considered significant when $p < 0.05$ with asterisks denoting the degree of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****; $p < 0.0001$).

Results

OS in the macrophages infected by H37Rv MTB

The control group's ROS, MDA, and CAT levels remained constant throughout 0, 4, 24, and 48 hours (Figure 1A–C). ROS and MDA increased at 4 hours and peaked at 48 hours in the H37Rv group, which was significantly higher than the control ($p < 0.05$). The CAT levels in the H37Rv group increased significantly compared to the control group at 4, 24, and 48 hours after infection ($p < 0.05$).

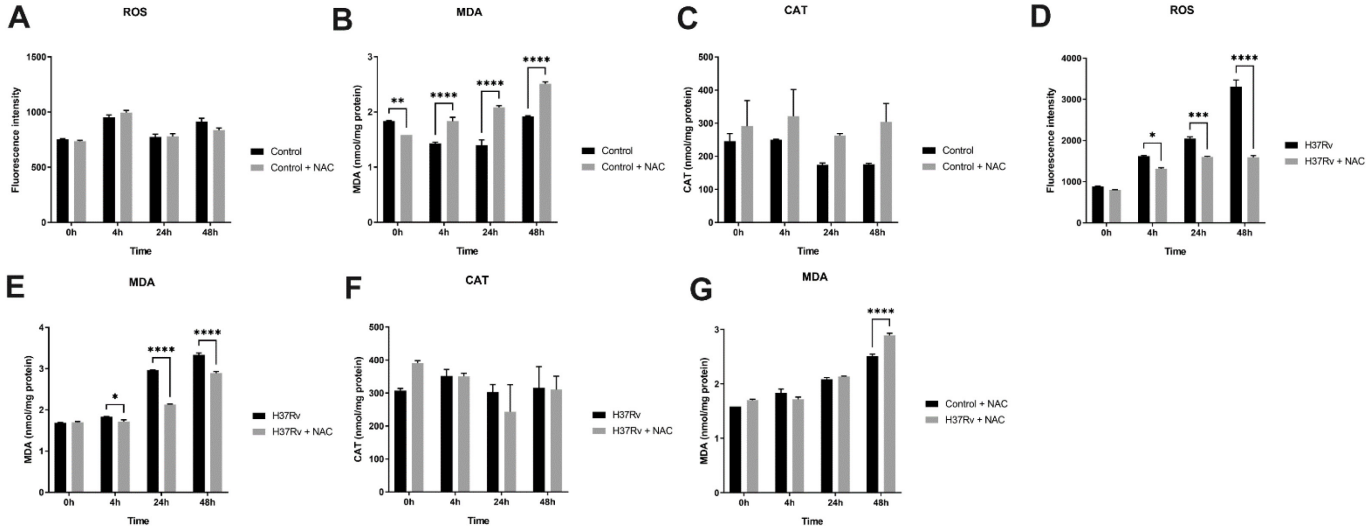
Cytotoxicity of NAC on the macrophages in different concentrations

The cell viability decreased significantly compared to untreated (0 mM NAC) ($p < 0.05$) only when macrophages was treated with 20 mM NAC.

Effect of NAC on the OS level of macrophages

MDA in the control + NAC group increased from 4 to 48 hours, to a level that was greater than the control group ($p < 0.05$), whereas ROS and CAT in the control + NAC group were comparable to the control group at each time point ($p > 0.05$, Figure 2A–C). Although ROS and MDA levels of the H37Rv + NAC group increased at 4, 24, and 48 hours, their levels were lower than those

Figure 2. Effects of N-acetylcysteine (NAC) on oxidative stress levels in macrophages infected and uninfected with H37Rv MTB. **A, D.** ROS levels; **B, E, G.** MDA levels; **C, F.** CAT levels.



Control group, uninfected and untreated with NAC; Control + NAC group, uninfected and treated with NAC; H37Rv group, infected with H37Rv MTB and untreated with NAC; H37Rv + NAC group, infected with H37Rv MTB and treated with NAC. ROS: reactive oxygen species; MDA: malondialdehyde; CAT: catalase; Values are presented as the mean ± SEM (n = 3). *: p < 0.05; **: p < 0.01; ***: p < 0.001; ****: p < 0.0001.

of the H37Rv group ($p < 0.05$; Figure 2D–F). However, CAT levels were not significantly different between the two groups ($p > 0.05$). At 48 hours, MDA levels of the control + NAC group were lower than those of the H37Rv + NAC group ($p < 0.05$, Figure 2G).

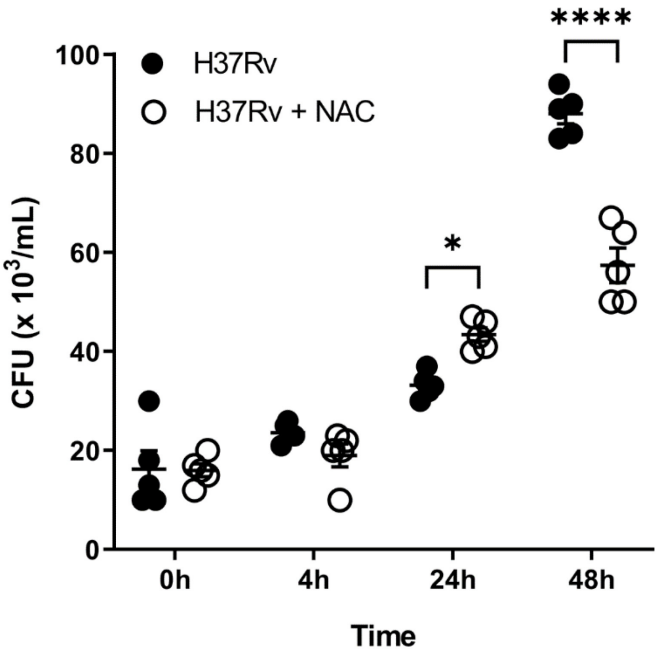
Effect of NAC on the intracellular MTB CFU of macrophages infected with H37Rv

The intracellular MTB CFU of the H37Rv + NAC group were comparable to those of the H37Rv group at 0 and 4 hours ($p > 0.05$), while the CFU of the H37Rv + NAC group were slightly higher than those of the H37Rv group at 24 hours ($p < 0.05$), and slightly lower than those of the H37Rv group at 48 hours ($p < 0.01$; Figure 3).

Effect of NAC on apoptosis of the macrophages infected with H37Rv

The apoptosis rate increased at 4 hours and reached a maximum at 48 hours in the H37Rv group, which was significantly higher than the control group ($p < 0.05$; Figure 4A–C). Compared to the control group, the apoptosis rate of the control + NAC group increased at 24 and 48 hours ($p < 0.05$). There was no significant difference in the apoptosis rate between the H37Rv + NAC group and the H37Rv group ($p > 0.05$). At 24 and 48 hours, the apoptosis rates of the control + NAC group were lower than those of the H37Rv + NAC group ($p < 0.05$).

Figure 3. The effect of N-acetylcysteine (NAC) (10mM) on the intracellular Mycobacterium tuberculosis (MTB) load of macrophages infected with H37Rv MTB.



H37Rv group, infected with H37Rv MTB and untreated with NAC; H37Rv + NAC group, infected with H37Rv MTB and treated with NAC. CFU: colony forming units. Values are presented as the mean ± SEM (n = 3). *: p < 0.05; **: p < 0.01.

Effect of NAC on autophagy of the macrophages infected with H37Rv

The LC3II/β-actin and P62/β-actin increased at 4 hours and reached a maximum at 48 hours in the H37Rv group, which was significantly higher than the control group ($p < 0.05$; Figure 4D–F). There was no change in autophagy levels between the control group and the control + NAC group ($p > 0.05$). At 48 hours, the LC3II/β-actin and P62/β-actin ratio of the H37Rv + NAC group was lower than that of the H37Rv group ($p < 0.05$).

Correlation between the effects of NAC on MTB mediated macrophages OS, intracellular CFU, apoptosis, and autophagy

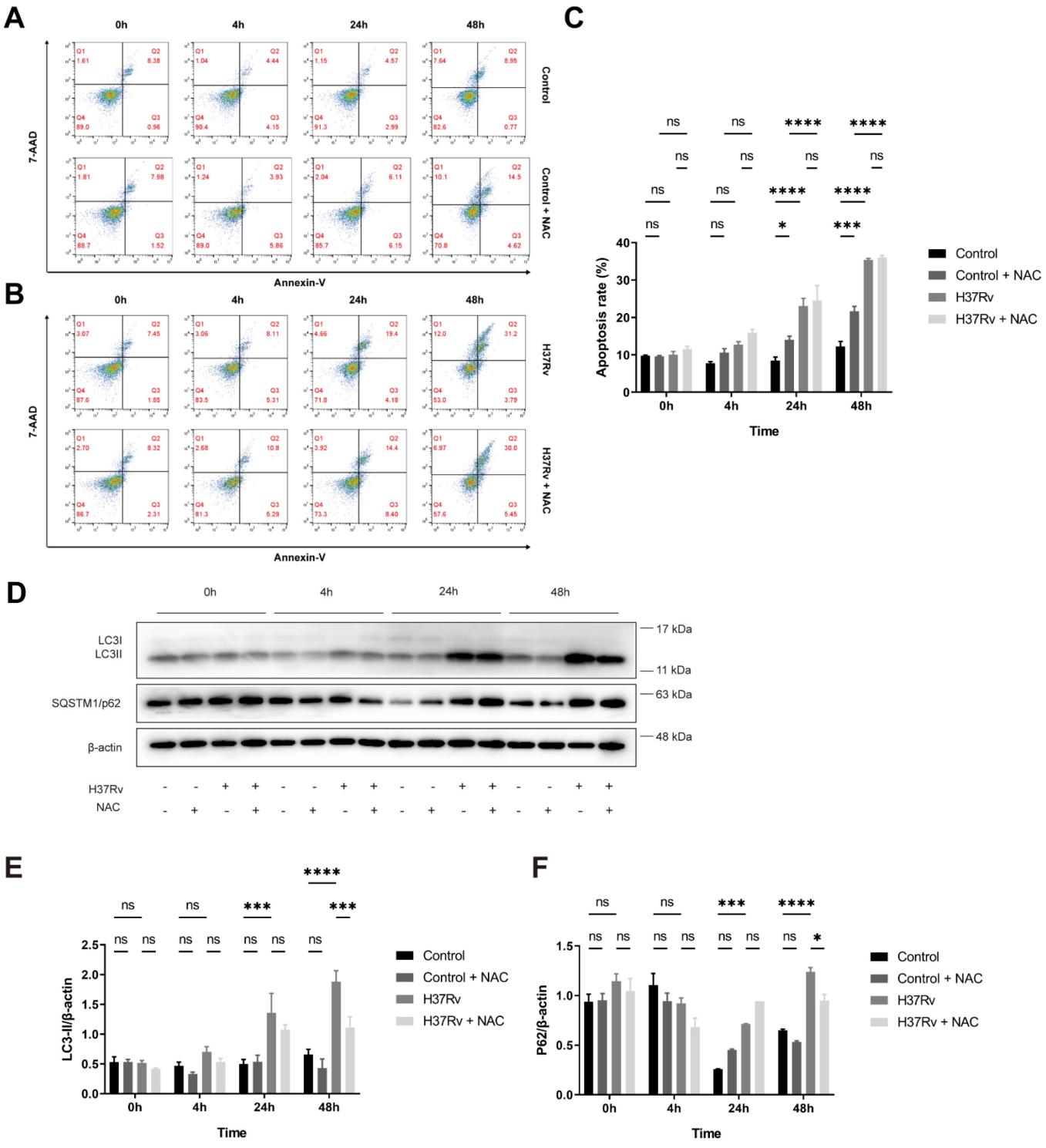
Figures 5A–D show the effects of NAC on MTB mediated macrophage OS, intracellular CFU, apoptosis, and autophagy at 0, 4, 24, and 48 hours. With the increase of CFU in macrophages of the H37Rv group, its ROS, apoptosis rate, and LC3II/ β- actin ratio increases. As the intracellular CFU of the H37Rv + NAC group decreased at 48 hours, its ROS and LC3II/ β-actin ratio also decreased, but the apoptosis rate remained unchanged.

Discussion

The production of ROS by MTB infected macrophages may be crucial for the treatment of tuberculosis [6]. In this study, we established a macrophage model infected with MTB and found that NAC not only reduced the ROS of macrophages infected with MTB, but also led to a decrease in the LC3II and P62. This study may provide a premise for a new mechanism of NAC in the treatment of tuberculosis.

This study showed that there were low levels of ROS in uninfected MTB macrophages. After H37Rv infection, ROS and MDA significantly increased, with a slight increase in CAT. These findings are consistent with another study that demonstrated that MTB infection induces macrophages to generate a substantial quantity of ROS and MDA, thereby leading to an oxidative imbalance [21]. A clinical study showed that the MDA level of tuberculosis patients was higher than that of the healthy group, which was similar to our study [22]. Additionally, we also found that MTB infection may increase CAT, which is less reported in tuberculosis.

Figure 4. Effect of N-acetylcysteine (NAC) on apoptosis and autophagy of macrophages infected with H37Rv. **A, B.** Apoptosis flow cytometry; Q3: early apoptosis; Q2: late apoptosis. **C.** Apoptosis rate levels; **D.** Western blot of LC3I, LC3II, and P62 expression levels; **E.** LC3II/β-actin ratio levels; **F.** P62/β-actin ratio levels.



Control group, uninfected and untreated with NAC; Control + NAC group, uninfected and treated with NAC; H37Rv group, infected with H37Rv MTB and untreated with NAC; H37Rv + NAC group, infected with H37Rv MTB and treated with NAC. β-actin, internal control protein; LC3I, LC3II, P62, autophagy related proteins; NAC: treated with N-acetylcysteine; H37Rv: infected with H37Rv MTB. Values are presented as the mean ± SEM (n = 3). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

Consequently, we analyzed the potential factors that contributed to this result. Firstly, MTB may produce CAT to degrade hydrogen peroxide (H_2O_2) to ensure its survival after being engulfed by macrophages [23]. Secondly, it is possible that glutathione peroxidase (GPX)'s ability to degrade H_2O_2 decreases in the absence of glutathione (GSH), and an increase in H_2O_2 may lead to an increase in CAT activity [24]. Finally, it may also be associated with MTB-mediated increase in macrophage ROS, leading to CAT secretion to degrade the H_2O_2 in it.

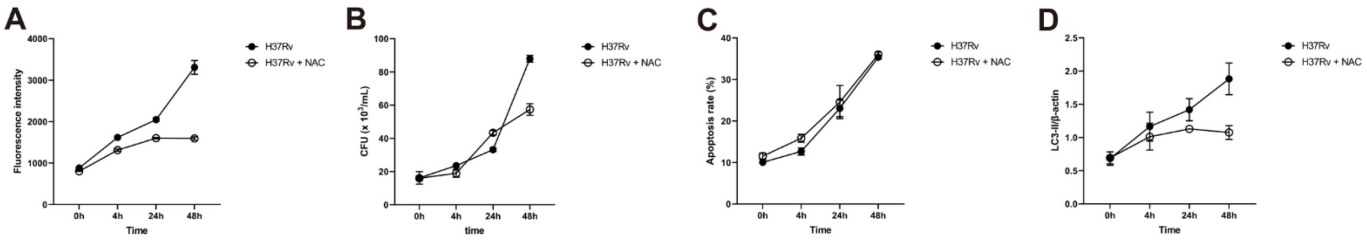
NAC can regulate the redox state in cells as a precursor of reduced glutathione and a direct scavenger of ROS [25]; so, it is frequently utilized as an antioxidant in OS-related research. It was determined that 20 mM NAC is toxic to macrophages. Therefore, the NAC dosage in our experiment was 10 mM. In the present investigation, MDA increased while ROS and CAT remained unchanged in the NAC group. The mechanism needs further research and the impact of NAC toxicity on lipid peroxidation cannot be ruled out.

The levels of ROS and MDA were lower in the H37Rv + NAC group compared to the H37Rv group, whereas CAT did not change significantly. At 48 hours, MDA levels of the control + NAC group were lower than those of the H37Rv + NAC group. The findings were similar to those reported by Amaral *et al*. [17], whose research demonstrated that the ROS level in the NAC group was lower than that in the infection group. However, it did not fully offset the increase in ROS caused by infection. It is comparable to another OS-related study [26], which may be related to NAC administration dosage. The findings of our research on CAT differ from the previous study. This may be because MTB secretes CAT into macrophages to ensure their survival [27].

There is evidence to suggest that NAC can affect the bacterial activity of eukaryotic host cells and reduce the content of MTB in macrophages [28]. In this study, the CFU of the H37Rv + NAC group was higher than that of the H37Rv group at 24 hours, and lower than that of the H37Rv group at 48 hours. Early NAC treatment did not affect the burden of MTB in macrophages, possibly because the effect of NAC on macrophages and their intracellular MTB requires a certain amount of action time. However, the effect of the NAC dose cannot be ruled out. Further work is needed to determine the mechanism of NAC's antibacterial effect.

Previous study has demonstrated that ROS produced by mitochondria can serve as a second messenger to regulate cell apoptosis [29]. The apoptosis rate of the H37Rv group in this study was higher than that of the control group, and its trend was consistent with intracellular CFU and ROS (Figure 5A–C). It is suggested that MTB infection of macrophages can stimulate an increase in their apoptosis rate, in order to kill the MTB in macrophages through apoptosis. In addition, ROS may also be one of the reasons for the increased apoptosis rate of macrophages. Several studies [30,31] demonstrated that MTB could activate multiple apoptosis pathways and that OS may participate in MTB-induced apoptosis. However, the apoptosis rates of the H37Rv + NAC group at various time points were similar to those of the H37Rv group. At 24 and 48 hours, the comparison between control + NAC group and H37Rv + NAC group was similar to the comparison between the H37Rv group and the H37Rv + NAC group. This indicated that NAC had no significant effect on the apoptosis of macrophages infected with MTB. It may be because other oxidative metabolites such as MDA maintain high levels, or because intracellular CFU remains high.

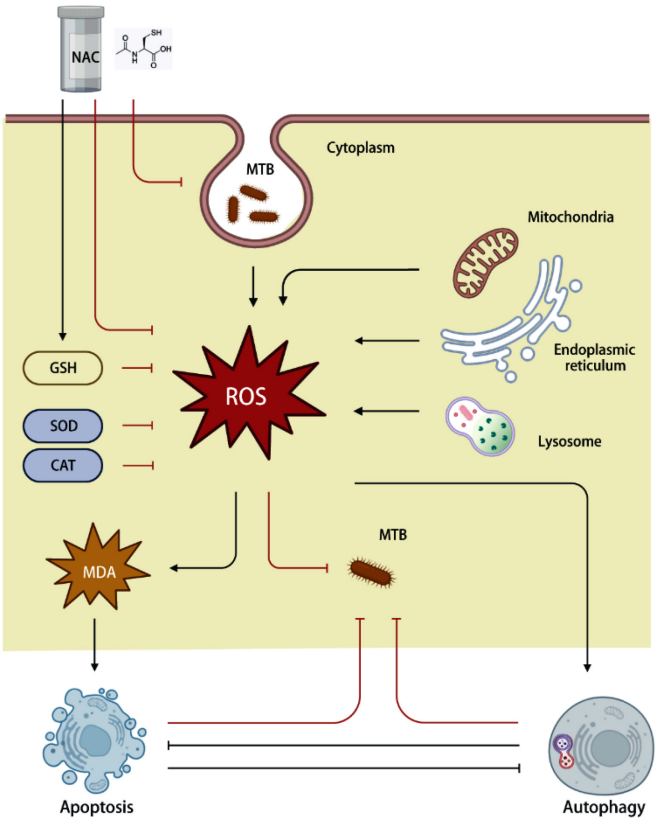
Figure 5. The correlation between the effects of NAC on MTB mediated macrophage OS, intracellular CFU, apoptosis, and autophagy. **A.** ROS levels; **B.** CFU levels; **C.** apoptosis rate levels; **D.** LC3II/β-actin ratio levels.



Control group, uninfected and untreated with NAC; control + NAC group, uninfected and treated with NAC; H37Rv group, infected with H37Rv MTB and untreated with NAC; H37Rv + NAC group, infected with H37Rv MTB and treated with NAC. β-actin, internal control protein; LC3I: LC3II: autophagy related proteins; NAC: treated with N-acetylcysteine; H37Rv: infected with H37Rv MTB. MTB: *Mycobacterium tuberculosis*. Values are presented as the mean ± SEM (n = 3).

To investigate the effect of NAC on autophagy of macrophages infected with MTB, we conducted immunoblotting on LC3 and P62. Autophagy can be triggered in cells under conditions of starvation, hypoxia, endoplasmic reticulum stress, and radiation [32,33]. In the present study, the macrophages infected with MTB have been observed to result in a notable upregulation of LC3II/β-actin and P62/β-actin at 24 and 48 hours compared to the control group. A previous study suggested that P62 interacts with LC3 and transports the altered protein to degradation through autophagy [34]. Therefore, our results suggest that autophagy may increase in macrophages infected with MTB. However, the excessive increase of autophagosomes leads to the accumulation of P62, thereby inhibiting autophagic flow. NAC treated uninfected macrophages showed no changes in LC3II/β-actin and P62/β-actin. NAC reduced LC3II/β-actin and P62/β-actin of macrophages infected with MTB at 48 hours. NAC may weaken the production of autophagy within macrophages, and also reduce the accumulation of P62 caused by a large amount of MTB. This may be related to a decrease in intracellular CFU and a decrease in ROS and MDA (Figure 5A, B, D). A previous study suggested that ROS was the main intracellular signal transducer responsible for maintaining autophagy [35]. There are also studies that indicated that OS could affect the regulatory mechanisms of autophagy and cell survival [36]. Autophagy has an inhibitory effect in the early stage of MTB infection [10]. There is limited research on the effect of NAC on macrophage autophagy, and further exploration is needed.

Figure 6. The cell membrane, mitochondria, endoplasmic reticulum, and lysosomes of macrophages produce ROS under MTB stimulation. NAC is an antioxidant that can inhibit ROS production by regulating GSH, and also has anti-tuberculosis effects. In addition to its own anti MTB effect, ROS also affects macrophage apoptosis and autophagy, thereby inhibiting MTB. Ultimately, the clearance of intracellular MTB can lead to a decrease in ROS.



NAC: N-acetylcysteine; MTB: *Mycobacterium tuberculosis*; ROS: reactive oxygen species; MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; CAT: catalase. This image was created using BioRender.com.

As shown in Figure 6, oxidative stress has complex crosstalk with host autophagy and apoptosis. Macrophages infected with MTB produce ROS. In addition to its anti-MTB effect, ROS also affects macrophage apoptosis and autophagy. NAC not only has antioxidant effects, but also has anti MTB effects. This study suggests that NAC may have the ability to regulate macrophage autophagy and apoptosis, which may be related to the bactericidal effect of NAC. The above evidence might provide a basis for in vitro experiment of NAC in adjuvant treatment of tuberculosis patients.

This research was limited to in vitro experiments and contained fewer indicators of apoptosis and autophagy. In future studies, we will select additional apoptosis and autophagy indicators and conduct in vivo experiments to obtain more specific data.

Conclusions

MTB infection can lead to an increase in macrophage OS and cell apoptosis, resulting in an increase in autophagy related protein LC3II and accumulation of P62. However, after treatment with NAC (10 mM), the growth of MTB in macrophages is inhibited, and OS, LC3II, and P62 are reduced. The antioxidant effect and inhibitory effect of NAC on MTB are related to MTB mediated macrophage OS and autophagy.

Funding

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Data availability

Data generated or analyzed during this study are provided within the published article.

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