Combination of N-acetyl-cysteine with Clarithromycin against *Mycobacterium avium* infection

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Background

Incidence of pulmonary nontuberculous mycobacterial disease (PNTMD) is reportedly increasing globally. In Japan, according to Ho Namkoong et al.(1), incidence rate for PNTMD was reported to be 14.7 cases per 100,000 person-years, which exceeded the incidence rate for tuberculosis (12.9 cases per 100,000 person-years) in 2014. Among PNTMD, M. avium complex (MAC; the combination of *Mycobacterium avium* and *Mycobacterium intracellulare*) infection is most common in Japan, as is the most case throughout the world. Drug therapy for MAC disease involves multiple drugs and risk of adverse drug reactions and/or toxicities is relatively high. In addition, the optimal therapeutic regimen has yet to be established. In search of therapeutic agent to augment the treatment of MAC infection, we focused on N-acetyl-cysteine (NAC) .

NAC is widely used in patients with chronic pulmonary diseases. In previous studies, its anti-microbacterial and anti-mycobacterial effects have been reported. Among its effect in mycobacteria, it has been mainly studied in *Mycobacterium tuberculosis* (MTB) (2,3). Here, we examined whether NAC has antibiotic activity against *Mycobacterium avium* (*M. avium*).

Methods

The anti-mycobacterial effect of NAC was assessed in JCM 15430 *M. avium* strain infected A-549 (human lung epithelial cells) and MH-S (mouse alveolar macrophages) . These cells were infected with *M. avium* at multiplicity of infection of 10 for 1 h, washed and then cultivated for 5 days. Bacterial uptake was evaluated at 5 days of cultivation. For NAC treatment group, 5% FBS medium with 10mM of NAC was used as culture medium. For L-Carbocisteine treatment group, 5% FBS medium with 10mM of L-Carbocisteine was used as culture medium.
Bacterial numbers were determined by serial dilution of bacterial cultures and cell lysates plated on Middlebrook 7H10 agar (Difco) supplemented with 10 % oleic acid/ albumin/dextrose/catalase (OADC; Difco). CFU numbers were determined after 2 weeks of incubation at 37 °C in 5 % CO_2. Total RNAs were isolated from infected cells and expression of cytokines and antimicrobial peptides was examined by qRT-PCR.

We also tested its effect in combination with clarithromycin in vivo. Female BALB/c mice were infected intranasally with 10^6 CFU of *M. avium*, and were treated with NAC (400 mg/kg) .

To assess the effect of NAC in combination with clarithromycin, mice were treated with NAC (400 mg/kg), clarithromycin (100 mg/kg) or both; NAC (400 mg/kg) and clarithromycin (100 mg/kg) by gavage daily for 6 days. On day 7 of infection, lungs were harvested and CFU, expression of cytokines and antimicrobial peptides was examined by qRT-PCR and RT² Profiler™ PCR Array Mouse Antibacterial Response; catalog number PAMM-148Z;QIAGEN .

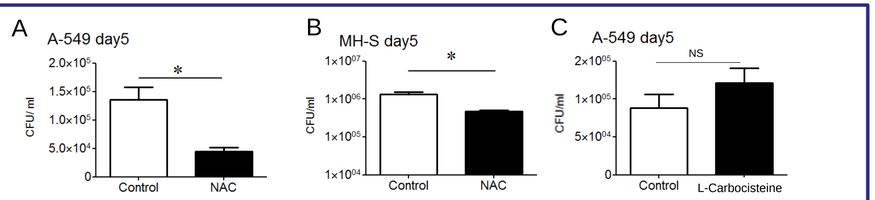

Fig. 1: Survival of *M.avium* treated with NAC or L-Carbocisteine

Significant reduction in mycobacterial load was observed for the NAC treated group compared to untreated group, both in A-549 (A) and MH-S (B) (*p <0.05). There was no significant difference in mycobacterial load between L-Carbocisteine treated and untreated group (C).

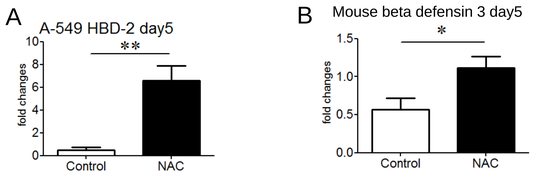

Fig. 2: NAC treatment results in a significant increase in mRNA expression levels of Human beta defensin-2 (HBD-2) and of Mouse beta defensin 3

Total RNAs were isolated from *M.avium* –infected cells (A-549: A, MH-S: B) in the presence or absence of NAC. Significant increase in mRNA expression levels of HBD-2 in A-549 (A), along with increase in mRNA expression levels of murine homologue of HBD-2; Mouse beta defensin 3 in MH-S (B) was observed (*p <0.05; **p <0.01)..

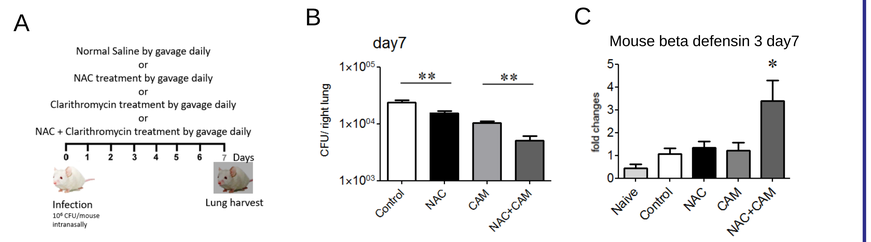

Fig. 3: NAC treatment results in a significant reduction of mycobacterial loads in the lungs from mice infected with *M. avium*

A. BALB/c mice were infected intranasally with 10^6 CFU of *M.avium* strain. Mice were treated or not with NAC (400 mg/kg)) or clarithromycin (100 mg/kg) or both by gavage daily for 6 days.
B. On day 7, mice were euthanized and lungs were harvested. CFU values in lungs were determined as described in Methods. Data represent individual values and means ± SD from a total of 7 animals per group (**p <0.01).
C. mRNA expression levels of Mouse b-defensin 3 significantly increased when treated with NAC and clarithromycin combination therapy (*p <0.05).

Results

NAC treatment of *M. avium* infected A-549 and MH-S resulted in a significant reduction of mycobacterial load (p=0.014 and p=0.014)) (Fig.1 A, B) . In contrast, when treated with another mucolytic agent, L-Carbocisteine, there was no reduction of mycobacterial load (Fig. 1 C).

mRNA expression levels of Human beta defensin-2 (HBD-2) and of Mouse beta defensin 3; homologue of HBD-2 significantly increased in NAC treated group (p=0.009 and p=0.012) (Fig. 2).

In vivo, NAC treatment resulted in a significant reduction of mycobacterial load in the lungs of *M. avium* infected mice (p=0.007)(Fig.3 B). When in combination with clarithromycin, we also had an additional reduction (vs. clarithromycin monotherapy; p=0.001)(Fig. 3 B).

mRNA expression levels of Mouse beta defensin 3 significantly increased when treated with NAC and clarithromycin combination therapy (Fig. 3 C). Otherwise, there were no significant changes in mRNA expression of cytokines nor other antimicrobial peptides, e.g. cathelicidin antimicrobial peptide, other Mouse beta defensins (1,2, and 4) and other antimicrobial peptides which can be analyzed with the previously mentioned PCR array kit.

Discussion & Conclusion

This study demonstrated a potent anti-mycobacterial effect of NAC against *M. avium*. Interestingly in our study, expression level of HBD-2 increased in NAC containing therapy group.

HBD-2 is a cationic antimicrobial peptide that exhibits a wide range of antimicrobial activity against viruses, bacteria, and fungi. Its gene expression has been identified in various human epithelia including lungs and trachea (4,5). In MTB infection, HBD-2 has been shown to control bacterial growth and has chemotactic activity (5). Thus, increase in expression level of HBD-2 may be one of the possibility on how NAC is involved in anti-mycobacterial effects on *M. avium*.

NAC exhibits potent anti-mycobacterial effects and may limit *M. avium* infection. In addition with clarithromycin, it showed additive effect in reduction of mycobacterial loads. As NAC is already widely used in clinical medicine, it may can be an additional option in treating *M. avium* infected patients in future, along with its classical drug regimens containing clarithromycin.

References

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