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Combined Treatment with Tauroursodeoxycholic Acid and SCD Probiotics Reduces Oxidative Stress in Lung Tissue of Aged Rats

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Abstract

Aging is associated with an increased level of oxidative stress, resulting from an elevated production of reactive oxygen species, which can lead to cellular and tissue damage, particularly in the lungs. In this study, it was aimed to evaluate the effects of tauroursodeoxycholic acid (TUDCA) and SCD Probiotics, both individually and in combination, on malondialdehyde (MDA), advanced oxidation protein products (AOPP), and myeloperoxidase (MPO) levels as oxidative stress markers in the lung tissue of aged Sprague-Dawley rats. The results showed that TUDCA significantly decreased MDA and AOPP levels, suggesting its antioxidant activity. SCD Probiotics also demonstrated a reduction in AOPP levels, highlighting their immunomodulatory and antioxidant effects. Furthermore, the combined treatment of TUDCA and SCD Probiotics resulted in a significant reduction in both MDA and AOPP levels, as well as a notable decrease in MPO activity. This suggests a synergistic interaction that enhances the antioxidative and anti-inflammatory properties of the individual treatments. These findings support the therapeutic potential of TUDCA and SCD Probiotics in mitigating oxidative damage in aging lung tissues, suggesting that their combined use may be a promising approach to combat age-related oxidative stress. Further research is needed to investigate these effects in various models and long-term applications.

1. Introduction

Aging is a biological process that involves a gradual decline in the functionality of cells and tissues, leading to an increased risk of age-related chronic diseases and mortality [1,2]. The aging process is influenced by numerous intricate and significant pathways associated with chronic oxidative stress resulting from excess levels of reactive oxygen species (ROS) [1]. ROS have been proposed as one of the contributing factors to aging due to their potential to induce oxidative damage to DNA, proteins, and lipids [3]. ROS are molecules derived from molecular oxygen that can have both beneficial and harmful effects. While ROS are byproducts of normal cellular metabolism and help maintain redox homeostasis, excessive ROS production leads to oxidative stress [4]. ROS, previously believed to come only from mitochondrial metabolism, however, it is now known that ROS are also produced by other cellular sources such as NADPH oxidases, neutrophils, monocytes, xanthine oxidases, cytochrome P450 enzymes, lipoxygenases, nitric oxide synthases, and myeloperoxidase [5,6].

Myeloperoxidase (MPO) is a critical enzyme of neutrophils, responsible for producing potent oxidants, such as hypochlorous acid (HOCl), which act as a bactericidal system against invading organisms. However, the uncontrolled generation of oxidants leads to lipid peroxidation and protein oxidation, resulting in cellular and tissue damage [7]. Lipid peroxidation is a process that involves removing a hydrogen from a carbon and adding an oxygen molecule by attacking carbon-carbon double

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bonds in lipids through reactive species. This process results in the generation of products such as malondialdehyde (MDA) and 4-hydroxynonenal [8]. Additionally, lipid peroxidation impacts proteins, causing structural changes that can impair their enzymatic functions [9]. Advanced oxidation protein products (AOPP), which are dityrosine-containing protein cross-linking products, serve as an indicator of oxidative stress and are correlated with the degree of monocyte activation, levels of dityrosine, advanced glycation end products, and inflammatory cytokines [10].

Age-related changes, such as telomere shortening, mitochondrial dysfunction, and increased oxidative stress, contributes to the inability of lung cells to maintain basic homeostasis [11]. Tauroursodeoxycholic acid (TUDCA) is a hydrophilic bile acid formed by the conjugation of the amino acid taurine with ursodeoxycholic acid [12]. It exhibits several properties, including antioxidation, anti-inflammation, anti-apoptosis, and neuroprotection [13]. The respiratory and gastrointestinal systems are closely interrelated with regard to their physiology and pathology [14]. In recent years, evidence has emerged on how probiotics and prebiotics, accepted as immunomodulators and gut health stimulators, could promote lung health [15]. The aim of the present study was to investigate the oxidative damage in aging lung by evaluating malondialdehyde, AOPP, and myeloperoxidase, and also the effect of probiotics and TUDCA on these oxidative damage markers.

2. Material and Method

2.1. Animals

Male Sprague-Dawley rats aged 24 months were enrolled in the study. The animals were divided into four groups. First group was the control group with no treatment, the second group received probiotics, the third group received TUDCA, and the fourth group received TUDCA in combination with SCD Probiotics supplementation (7 rats in each group). Each group received treatment for seven days. The rats were fed ad libitum with a standard rodent diet. TUDCA was administered intravenously at a dose of 300 mg/kg via the tail, while the probiotic supplement was given orally by gavage at a dose of 3 mL (1 x 108 CFU) per day [16]. The probiotic product used in the study, which contained 11 different strains including *Bacillus subtilis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactococcus lactis, Saccharomyces cerevisiae, and Streptococcus thermophilus* species, was marketed by SCD Probiotics company (Essential Probiotics XI - 500 ml H.S. Code: 2206.00.7000). After the treatment, the animals were euthanized one day later by ether treatment, and the lung tissues were extracted, immediately frozen on dry ice, and stored at 80°C until analysis. The animals were housed according to standard animal care protocols, and the study was approved by the Ethics Committee (approval number: 2022/03) of the Saki Yenilli Experimental Animal Production and Practice Laboratory.

2.2. Tissue Preparation

Lungs were taken out and rinsed with cold phosphate buffered saline (PBS). They were then homogenized in five volumes of cold 10 mM phosphate buffered saline (PBS, pH 7.4) containing protease inhibitor (PIC002, BioShop, Canada) and 0.5% Triton X-100 using a tissue homogenizer in an ice bath. Afterwards, the homogenates from the lung tissues were spun at 5,000xg for 10 minutes at 4°C. The supernatant was then collected and stored in aliquots at -80°C until analysis.

2.3. Protein Determination

The protein content in the supernatants was measured by the Bradford method using bovine serum albumin as standard [17].

2.4. Determination of Myeloperoxidase Activity

Measurement of myeloperoxidase (MPO) activity was carried out using a spectrophotometer. This was done by mixing the samples with 50 mM PBS, adjusted to pH 6.0, and containing o-dianisidine (0.167 mg/ml) and hydrogen peroxide (0.0005%). The absorbance was then measured at 460 nm for 5 minutes. The MPO activity was expressed in units per milligram of protein, as described by Bradley et al. (1982) [18].

2.5. Measurement of Malondialdehyde

Malondialdehyde, an indicator of lipid peroxidation was determined by monitoring thiobarbituric reactive substances (TBARS) formation. In brief, the samples were reacted with 20% trichloroacetic acid and 0.67% thiobarbituric acid, and then heated in a boiling water bath at 100°C. After cooling, the absorbance was

measured at 532 nm. The results were expressed as nmol/mg protein [19].

2.6. Measurement of Advanced Oxidation Protein Products

Advanced oxidation protein products, an indicator of oxidative damage to proteins, were assessed by measuring dityrosine-containing and cross-linking protein products. The AOPP levels were determined using the method developed by Witko-Sarsat and colleagues [20]. Specifically, the samples were diluted to a concentration of 20 mM with PBS (pH 7.4), followed by the addition of potassium iodide and acetic acid. The absorbance was then measured at 340 nm, and the values were expressed as nmol/mg protein.

2.7. Statistics

Statistical analyses and graph plotting of the results were performed using GraphPad Prism 9 (GraphPad, USA) to evaluate the experimental data. The data were analyzed using One-way ANOVA. Tukey's multiple comparisons test was performed for the difference between each pair of means. The statistical analyses were presented as mean ± standard error of the mean (SEM). The level of significance was indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results and Discussion

The current study focuses on investigating the dynamics of oxidative stress in aging lung tissue, and evaluating the potential of interventions such as TUDCA and SCD Probiotics to mitigate the detrimental effects of oxidative damage. With advancing age, there is a marked increase in the production of ROS, which significantly contribute to cellular and tissue dysfunction through complex pathways of oxidative damage [1,2]. This accumulation of ROS is closely linked to lipid peroxidation and protein oxidation, both of which are identified as pivotal factors in the aging process of cellular systems [3]. Recent studies have further validated these observations, demonstrating that enhanced oxidative stress is associated with a decline in mitochondrial function and an increase in cellular senescence, thereby exacerbating age-related pathologies [21,22]. Moreover, researchers elucidate how oxidative modifications to cellular proteins not only reduce their functional capacity but also trigger inflammatory responses that further deteriorate tissue integrity [9,23,24,25]. The strategic use of TUDCA with antioxidant properties and the SCD Probiotics which balance gut microbiota, could play a crucial role in countering these oxidative processes and ameliorating the impacts of aging on lung tissues [16,26,27]. Since aging is a multifactorial and complex process, this combined treatment may offer a therapeutic approach to improve cellular resilience and overall lung health.

3.1. Effects of TUDCA, SCD Probiotics and Their Combined Treatment on Lung MDA Levels

The increase in lipid content with age may lead to higher lipid peroxidation. Additionally, increased ROS production has been reported with aging. Therefore, lipid peroxidation can be used as a marker for early and reversible tissue damage, as well as a reduction in antioxidant defenses. With aging, lipid peroxidation damage increased, and a notable decline in antioxidant defenses was reported in the previous studies [9,28,29]. Elevated MDA levels cause damage to macromolecules, leading to alterations in cellular structure and function. This damage worsens oxidative stress and inflammation, which further increases MDA generation. This process contributes to the progressive decline in physiological functions and the development of diseases. High plasma MDA levels increase the burden on the kidneys and promote a cyclical process of oxidative stress, which contributes to the decline in kidney function with age [30].

The findings of this study reveal a significant reduction in the MDA levels within lung tissue following treatments with TUDCA, SCD Probiotics, and their combined use, compared to the controls ($p=0.03$, $p=0.012$, $p=0.007$, respectively). This notable decrease in MDA levels underscores the potential of these agents in mitigating oxidative stress and protecting pulmonary tissue. In particular, the combined treatment group demonstrated a more pronounced reduction in MDA levels compared to treatments with either combined TUDCA or SCD Probiotics alone, potentially indicating a synergistic effect (Figure 1). Previous studies reported TUDCA treatment supports functional recovery by alleviating oxidative stress, inflammatory response and apoptosis in different experimental models [12,31,32]. In addition, growing evidence shows that probiotics provide beneficial antioxidative effects by scavenging ROS and activating specific enzyme activities [33,34,35]. Consistent with previous findings, our results suggest that the administration of TUDCA and

probiotics may help to reduce lipid peroxidation, thereby slowing cellular aging in the lungs.

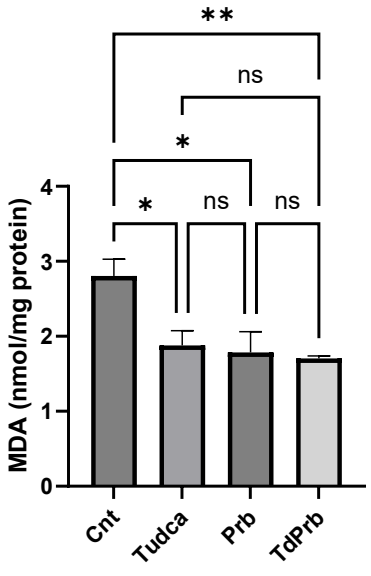

Figure 1. Effects of tauroursodeoxycholic acid, SCD Probiotics and combined treatment on lung malondialdehyde (MDA) levels. All data are shown as Mean ± SEM and the one-way ANOVA was used to compare the means of groups. Statistically significant differences are indicated in the following way: ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$. Cnt (control), Tauroursodeoxycholic acid (TUDCA), Prb (SCD Probiotics) and TdPrb (combined treatment)

3.2. Effects of TUDCA, SCD Probiotics and Their Combined Treatment on Lung AOPP Levels

In the study, it was found that TUDCA lowered the levels of AOPP in lung tissue due to its antioxidant properties ($p=0.04$) (Figure 2). TUDCA has been extensively documented for its potent antioxidative properties, particularly in stabilizing mitochondrial functions and inhibiting apoptotic pathways, which are essential for maintaining cellular integrity in aging tissues. It enhances mitochondrial biogenesis and reduces the release of cytochrome c, which is crucial in preventing cell death. TUDCA promotes tissue regeneration and enhanced functional recovery by decreasing oxidative stress, apoptosis, and inflammation in previous studies [12,13,36].

SCD Probiotics treatment resulted in lower AOPP levels, however, no statistically significant difference was found (Figure 2). Treatment with specific probiotics has been also associated with a decrease in AOPP levels, highlighting the crucial role of gut microbiota in modulating systemic oxidative stress and inflammation. This effect is thought to be mediated by the enhancement of gut barrier function and modulation of the gut-immune system interaction, leading to reduced systemic inflammatory markers and oxidative stress [37,38]. Lactic acid bacteria have been suggested as a promising source of antioxidants, offering potential benefits in alleviating the harmful effects of oxidative stress, a major contributor to the aging process [39]. Studies have demonstrated that lactic acid bacteria produce a range of antioxidants that help neutralize free radicals and diminish oxidative stress, which may enhance cellular health and support healthy aging [40,41]. In a randomized double-blind study, receiving of *Lactobacillus helveticus* resulted in a significant decrease in MDA and AOPP levels [42]. Furthermore, emerging studies emphasize the significance of gut microbial composition in influencing the host's antioxidant capacity, thereby providing a protective mechanism against oxidative damage [43]. In the combined treatment group, a reduction in AOPP levels was observed, emphasizing the synergistic interaction between TUDCA and SCD Probiotics ($p=0.02$) (Figure 2). It was concluded that the combined treatment is more effective than the application of either agent alone, suggesting that the concurrent use of these agents provides stronger protection against oxidative damage. In addition, these findings suggest that both TUDCA and probiotic treatments may be strategically used to mitigate the impacts of aging by enhancing antioxidative defenses and maintaining cellular and systemic homeostasis.

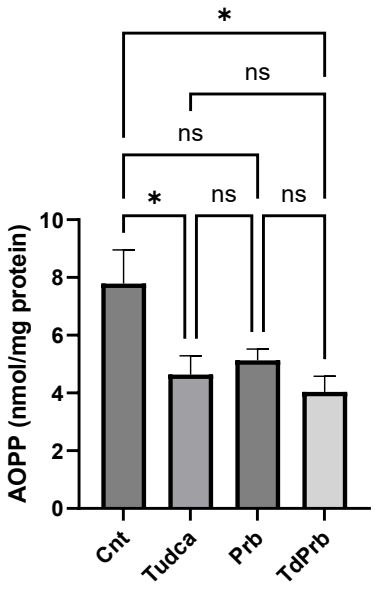

Figure 2. Effects of tauroursodeoxycholic acid, SCD Probiotics and combined treatment on lung advanced oxidation protein products (AOPP) levels. All

data are shown as Mean ± SEM and the one-way ANOVA was used to compare the means of groups. Statistically significant differences are indicated in the following way: ns $p > 0.05$; * $p < 0.05$. Cnt (control), Tauroursodeoxycholic acid (TUDCA), Prb (SCD Probiotics) and TdPrb (combined treatment)

3.3. Effects of TUDCA, SCD Probiotics and Their Combined Treatment on Lung Myeloperoxidase Activity

MPO plays a significant but complex role in the production of reactive oxygen species, specifically hypochlorous acid, which possesses strong bactericidal properties but can also contribute to cellular and tissue damage if not adequately controlled [7]. This dual nature of MPO underscores its importance in inflammatory and oxidative processes. Our study provides evidence that both TUDCA and SCD Probiotics effectively reduce MPO activity, which may act as a crucial protective mechanism against excessive oxidative stress within lung tissues ($p=0.0002$, $p=0.0006$, respectively) (Figure 3). This observation is consistent with recent findings that emphasize the anti-inflammatory and antioxidative properties of TUDCA, which has been shown to modulate inflammatory pathways and reduce oxidative stress in several models of disease [12,13,44]. The benefits of probiotics on systemic and localized inflammation are well documented [45,46,47,48]. Therefore, their potential may decrease MPO levels by modulating the gut-lung axis and thereby reducing pulmonary oxidative stress. These findings suggest that the targeted use of TUDCA and SCD Probiotics may offer therapeutic benefits in managing conditions characterized by elevated oxidative stress and inflammation. Further studies are needed to investigate these complex interactions between MPO activity, oxidative stress, and potential interventions.

One of the most significant findings of our research is the revelation of a synergistic effect when TUDCA and SCD Probiotics are administered together. This synergistic effect not only enhances the reduction of MDA and AOPP levels but also significantly decreases MPO activity ($p<0.0001$), suggesting that the combination of these treatments amplifies their individual antioxidative and anti-inflammatory effects. This finding is essential for developing therapeutic approaches that aim to minimize oxidative damage and promote cellular longevity in aging populations. Previous studies have reported the potential of probiotics in modulating systemic inflammation, which could contribute to lower MPO levels through the modulation of the gut-lung axis, thus reducing pulmonary oxidative stress [26,27]. Moreover, the application of synbiotics has been demonstrated to effectively decrease inflammation in both healthy and diseased states, suggesting a broad therapeutic potential [49]. These observations support the idea that the systemic modulation of inflammation by gut microbiota interactions is a promising approach for decreasing inflammatory markers and oxidative stress across various conditions [50].

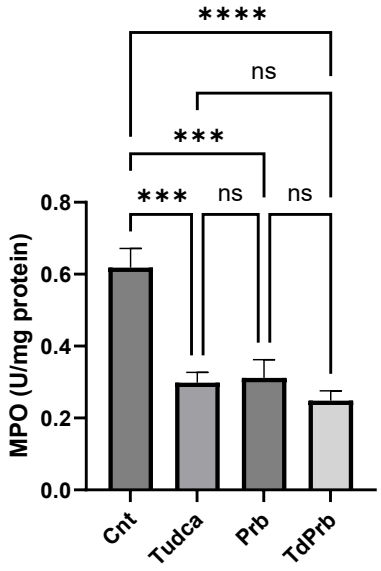

Figure 3. Effects of tauroursodeoxycholic acid, SCD Probiotics and combined treatment on lung myeloperoxidase (MPO). All data are shown as Mean ± SEM and the one-way ANOVA was used to compare the means of groups Statistically significant differences are indicated in the following way: ns $p > 0.05$; *** $p < 0.001$, **** $p < 0.0001$. Cnt (control), Tauroursodeoxycholic acid (TUDCA), Prb (SCD Probiotics) and TdPrb (combined treatment)

The study's outcomes reveal promising therapeutic pathways that warrant further exploration and optimization for the treatment or prevention of age-related degenerative diseases in lung tissue. Considering the complex nature of aging and the multifaceted roles of oxidative stress and inflammation, it is essential to conduct longitudinal studies to comprehensively understand the long-term consequences and potential side effects of these treatments. Furthermore, expanding the research to comprise various age groups and additional oxidative stress markers may provide deeper insights into the broader applicability of TUDCA and probiotics as preventive or therapeutic agents.

4. Conclusion and Suggestions

This study emphasizes the considerable potential of TUDCA and SCD Probiotics, both individually and collaboratively, to alleviate oxidative stress indicators in aging lung tissues. By reducing levels of malondialdehyde, advanced oxidation protein products, and myeloperoxidase activity, these treatments are effective in supporting cellular health and counteracting the detrimental effects of oxidative damage associated with aging. The synergistic effects observed with combined treatments particularly suggested that consolidated therapeutic approaches may offer improved protection against the progression of age-related pulmonary diseases. In the future, elucidating the mechanisms behind the effects of TUDCA and probiotics on the respiratory tract in diseased states will be crucial. Furthermore, this area of research is likely to become a key focus in pharmacology and clinical practice.

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Contributions of the authors

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

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