Kobe University Repository : Kernel

PDF issue: 2026-06-06

Budesonide/glycopyrronium/formoterol fumarate triple therapy prevents pulmonary hypertension in a COPD mouse model via NFκB activation

Suraya, Ratoe

(Degree)
博士（医学）

(Date of Degree)
2023-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲第8599号

(URL)
https://hdl.handle.net/20.500.14094/0100482347

※ 当コンテンツは神戸大学の学術成果です。無断複製・不正使用等を禁じます。著作権法で認められている範囲内で、適切にご利用ください。

（課程博士関係）

学 位 論 文 の 内 容 要 旨

Budesonide/glycopyrronium/formoterol fumarate triple therapy prevents pulmonary hypertension in a COPD mouse model via NFκB inactivation

ブデソニド/グリコピロニウム/ホルモテロールの３剤による吸入合剤は NFκB の不活性化を介して COPD マウスモデルの肺高血圧への進展を抑制する

神戸大学大学院医学研究科医科学専攻

呼吸器内科学

（指導教員：平田　健一　教授）

RATOE SURAYA

Background and purpose:

Chronic obstructive pulmonary disease (COPD) has been a major health problem, and one of the main complications that contributes to COPD-related death is the eventual development of pulmonary hypertension (PH), grouped into group 3 PH by WHO. Hypoxia-hypoxemia due to obstructed airflow is known to induce vascular remodeling in COPD-PH; however, unlike other classes of PH where specific drugs are available, few, drugs have been shown to successfully treat COPD-PH. Recently, a novel triple inhalation drug (inhaled corticosteroids/ICS, long-acting beta agonists/LABA, and long-acting muscarinic antagonists/LAMA) was shown to improve lung function in COPD patients. This triple therapy includes budesonide (B), glycopyrronium (G), and formoterol (F), which are the most widely used inhalation drugs and have been established as staples of COPD therapy. The clinical effects of this triple therapy have also been well established. Furthermore, the individual classes of drugs that constitute BGF therapy have been associated with positive effects in pulmonary vasculature cells, suggesting their potential benefit in treating PH, specifically COPD-PH. This include the cytokine-mediated inflammatory pathways via NFκB, a pathway controlling vascular tone and the subsequent remodeling process that is a common link between COPD and PH. In this study, we hypothesized that BGF combination therapy could act to prevent the development of pulmonary hypertension in COPD via NFκB-mediated inflammation pathway deactivation, and we sought to prove this hypothesis accordingly.

Methods:

We utilized one-time intratracheal elastase-induced emphysema mouse model and performed experiments in three phases illustrating COPD progression: inflammatory (1 day post-elastase), emphysema (3 weeks post-elastase) and PH (4 weeks post-elastase), while treatments of BGF combination and controls (vehicle, one-drug treatment with B, G, or F, and two-drug combinations of BF and GF) were started in prior to elastase instillation once (inflammatory phase), at day 7 (emphysema) three times weekly, or at day 14 (PH phase) three times weekly, respectively. Phenotype analyses were performed in each phase. These include bronchoalveolar lavage fluid (BALF) and tissue cytokine expression analysis in inflammatory phase, histological morphometric analysis in

emphysema phase, and right ventricular systolic pressure (RVSP) and histological vascular muscularization analysis in PH phase.

In vitro, A549 lung epithelial cells or isolated mouse lung endothelial cells (MLEC) were treated with TNFα with/without BGF treatment to analyze NFκB signaling and cytokine expression changes, while similar TNFα treatment in A549 cells with additional different combinations of drugs treatment (B, G, F, BF, GF, BGF) were performed to analyze the effectiveness of each drug combination to prevent cytokine expression.

Results:

First, in the inflammatory phase of *in vivo* study, we collected BALF from mice in all the experimental groups and performed cytological analysis of BALF smears. Cytology analysis of BALF revealed an increase in the total cell counts after elastase treatment, while treatment with G, F, BF, GF and BGF significantly decreased the elastase-induced increase in cell counts. Furthermore, a significant increase in the neutrophil percentage, with a subsequent decrease in the macrophage percentage were found after elastase treatment compared to PBS/vehicle treatment, while only GF and BGF treatments significantly ameliorated the neutrophil percentage increase. Real-time quantitative PCR analysis of the lung tissue mRNA from all groups revealed that the elastase-induced increase in the levels of IL-1β and CXCL2 were ameliorated in all of the treatment groups, including the BGF treatment group.

We then performed an analysis of the 21 days post-elastase emphysema phase. We first observed the peripheral oxygen saturation levels of the mice at Days 0 (prior to elastase instillation as baseline), 2, 9, and 16. While similar trends of saturation could be seen at days 2 and 9, at Day 16 after treatment commenced, the BGF/elastase group successfully reached an oxygen saturation level similar to that in the PBS/vehicle group. Further analysis of histological lung sections revealed a highly disrupted alveolar structure three weeks post-elastase instillation, yet improvement of the lung structure could be seen in the BGF-treated group, confirmed by semiautomatic mean linear intercept (MLI) measurements

Lastly, in the PH phase of our *in vivo* experiments, prior to termination at Day

28, echocardiography examination of the mice was performed, and the results revealed a significant decrease in the pulmonary artery acceleration time (PAAT) after elastase treatment, while BGF treatment successfully prevented it. At day 28, invasive hemodynamic analysis using right heart catheterization to measure RVSP showed the efficacy of BGF treatment in preventing the increase in the RVSP after elastase treatment. Consistently, right ventricular hypertrophy measurement using the Fulton index showed that BGF and BF treatment could prevent the development of right ventricular hypertrophy. Further, we observed thickened precapillary vessels after elastase treatment that was not observed in the BGF treatment group. We then confirmed that muscularization of the precapillary arteries indeed occurred in the PBS/elastase group, and it was ameliorated after the drug treatments, especially after BGF treatment, after double immunostaining of the lung sections using von willebrand factor and α-smooth muscle actin stainings.

We also aimed to prove that BGF could indeed affect this central inflammatory pathway. We performed *in vitro* experiments using lung epithelial A549 cells and isolated mouse lung endothelial cells (MLEC) treated with TNFαor vehicle, and simultaneously added BGF or vehicle control. Immunoblotting of NFκB p65 showed a marked increase in the levels of phosphorylated NFκB p65 after TNFα treatment, while the addition of BGF successfully abolished this phosphorylation in both cells. Additionally, we also analyzed whether there were differences in the production of cytokines after BGF treatment. Consistent with the immunoblotting results, TNFα induced increases in the mRNA levels of cytokines in both epithelial and endothelial cells, and these effects were successfully abolished by BGF treatment. Lastly, we found that among all drug combination groups, those containing budesonide (B, BF, BGF) were the ones most responsible in affecting the cytokine production, shown by the significant reductions in mRNA expression level of CXCL-8 and IL-6 compared to TNFα-treated A549 cells.

Discussion:

Although PH development is one of the major causes of morbidity and mortality in COPD patients, therapeutic options that can specifically address COPD-PH conditions are limited. We present here a novel strategy based on the inhalation of triple therapies

that could help treat this condition while providing molecular evidence about how such treatments might act on lung cells. It is interesting to see not only that BGF treatment is confirmed to be effective in both of the COPD phases (acute inflammatory and emphysematous phases) but also that it can be effective in preventing further development of PH.

Inflammation is a common mechanism in COPD and subsequent PH development, and it could also serve as a link between COPD and PH pathogenesis. BGF treatment is effective in preventing inflammation in epithelial cells, and it can also exert a direct effect in ameliorating proinflammatory responses in ECs, suggesting a possible common mechanism underlying the effect of BGF in treating this pathway. Multiple proinflammatory cytokines are affected by BGF treatment, pointing to a central effect of BGF in mediating inflammatory reactions. Indeed, we found that BGF exerted its effects through a similar mechanism involving the NFκB pathway in both A549 cells and MLECs. The transcription factor NFκB is known to trigger cellular inflammatory reactions after activation by triggering the mRNA expression of a host of proinflammatory cytokines Our study showed that BGF can directly ameliorate this pathway in both cell lines, suggesting that this compound may have a common molecular mechanism that can ultimately exert similar effects regardless of cell type. We did not observe any changes, however, to other PH and/or COPD-related pathways that could be related to the effect of BGF treatment. Further molecular studies are warranted to elucidate this mechanism.

One aim of our study was to analyze whether there is a preferable combination that can differentially affect lung cells in different phases. While BGF seemed to exert a synergistic effect in preventing proinflammatory reactions in all experimental phases that was not exerted by other drugs, interestingly, dual combination therapy did not exert a protective effect consistent with that of BGF in vivo, suggesting a synergystic effect between the three drugs. This could be explained by the interactions and synergism between steroids and β agonists in exerting anti-inflammatory effects, and between formoterol and glycopyrronium in affecting different pathways of anti-inflammatory and bronchodilating effects. Additionally, it is interesting to note that although the dual drug combinations showed tendencies of improving emphysematous and PH conditions, the

effects were relatively milder and less consistent than those of the BGF combination treatment. Moreover, the results from these three phases suggest that different drugs can positively affect COPD and COPD-PH conditions in different manners.

Further, we found that combinations that included budesonide had the strongest effects on the expression of proinflammatory cytokines, as expected for a potent anti-inflammatory agent. Notably, there was a decreasing trend in their expression with the addition of formoterol and a further reduction with the addition of glycopyrronium. The mutually beneficial interactions between steroids and β agonist agents could be the cause of this phenomenon, although the link with glycopyrronium is unknown. Still, the seemingly positive synergistic effect of the BGF combination warrants further studies in the future.

In our study, we not only confirmed the efficacy of BGF in COPD but also showed the additional benefit of BGF for COPD-PH conditions. The usage of the drug classes LAMA and LABA to specifically treat type 3 PH has not been widely studied in either basic or clinical settings. Corticosteroids, on the other hand, have been reported to be potentially effective in treating PH. Nevertheless, there is a notable lack of clinical data related to the usage of corticosteroids. As such, we hope that the results of our study will contribute to filling the knowledge gap on this specific topic.

In conclusion, the results of our study contributed to the increasing evidence that suggests the benefits of BGF treatment in COPD while also suggesting that BGF treatment could also be effective in preventing the occurrence of COPD-related PH. Further studies regarding the clinical efficacy of BGF in COPD-PH could potentially provide a novel way to treat COPD-PH patients in the future.