Montelukast improves bronchopulmonary dysplasia by inhibiting epithelial-mesenchymal transition via inactivating the TGF-β1/Smads signaling pathway

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Abstract. The present study investigated the role of montelukast (MK) during the progression of bronchopulmonary dysplasia (BPD) and the underlying mechanism of action. A rat model of BPD was induced by hyperoxia and subsequently, the rats were treated with 10 mg/kg MK. On day 14 post-hyperoxia induction, lung function was assessed by detecting the mean linear intercept (MLI; the average alveolar diameter), the radial alveolar count (RAC; alveolar septation and alveologenesis) and the lung weight/body weight (LW/BW) ratio. Type II alveolar epithelial (AEC II) cells were isolated from normal rats to investigate the mechanism underlying the effect of MK on BPD *in vitro*. Western blotting and reverse transcription-quantitative PCR were performed to measure the expression levels of surfactant protein C (SP-C), E-cadherin, N-cadherin, Vimentin, collagen I (Col I), matrix metallopeptidase (MMP)1/3, transforming growth factor (TGF)-β1 and Smad3. MK significantly reduced the MLI and the LW/BW ratio, and increased the RAC of the BPD group compared with the control group. MK upregulated the expression of SP-C and E-cadherin, and downregulated the expression levels of N-cadherin and Vimentin in the lung tissues of the rat model of BPD, as well as in TGF-β1- and hyperoxia-induced AEC II cells. In addition, MK reduced the expression of Col I, MMP1, MMP3, TGF-β1 and Smad3 in the lung tissues of the rat model of BPD, as well as in TGF-β1- and hyperoxia-induced AEC II cells. The present study demonstrated that MK improved BPD by inhibiting epithelial-mesenchymal transition via inactivating the TGF-β1/Smads signaling pathway.

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Introduction

Bronchopulmonary dysplasia (BPD) is a common chronic respiratory disease in preterm infants. Among preterm infants with a gestational age <28 weeks, the incidence of BPD is ≥50% and the mortality rate is ~64%. In addition, the readmission rate of surviving preterm infants in the first year is >50% and the occurrence of neurological dysplasia is 2-3 times higher compared with normal infants (1-3). Clinically, classical BPD primarily manifests as oxygen therapy dependence, which can result in pulmonary infection, pulmonary hypertension, heart failure and other complications (4-6). BPD has become a major disease that is responsible for death and poor prognosis in premature infants, seriously affecting their long-term quality of life. BPD is harmful, but there is no effective treatment strategy at present. It has been hypothesized that glucocorticoids display a certain therapeutic effect on BPD (7); however, based on the existing clinical evidence, the optimal dosage scheme cannot be obtained. Moreover, glucocorticoids are associated with serious adverse reactions, including inhibition of the development of the nervous system and an increased incidence of cerebral palsy (8,9). Therefore, identifying a novel, safe and more effective BPD treatment strategy is important.

Montelukast (MK) is a common leukotriene receptor antagonist with anti-inflammatory and antifibrosis effects, which is typically used for asthma, allergic rhinitis and other respiratory diseases (10). Recently, a number of studies have reported that MK also displays a protective effect in rheumatoid arthritis, ischemia reperfusion injury and Parkinson's disease (11-13). Additionally, it has been reported that increased leukotriene levels are associated with BPD. Rupprech *et al* (14) treated preterm infants with BPD with MK, and the results indicated that MK significantly improved the survival rate of children with advanced severe BPD and reduced the duration of mechanical ventilation, indicating improved efficacy and a reduced risk of adverse reactions (15). However, Hou *et al* (16) demonstrated that compared with the normoxic control group, hyperoxia significantly increased the level of cysteinyl leukotrienes in bronchoalveolar lavage fluid of newborn rats, and MK treatment did not improve the

inhibition of hyperoxia-induced alveolarization. Therefore, further investigation into the mechanism underlying the effects of MK during BPD is required.

Numerous signaling pathways that are crucial to lung development have been studied, including the transforming growth factor (TGF)-β signaling pathway (17,18). TGF-β signaling can negatively regulate the branch and interval of lung development, and has been demonstrated to be essential for normal lung development through the regulation of endothelial cell (EC) differentiation, growth, migration, senescence, and extracellular matrix (ECM) production (19,20). TGF-βs consist of three proteins, TGF-β 1, 2, and 3, and TGF-β1 activates the Smad pathway and non-Smad pathways such as mitogen-activated protein kinase, phosphoinositide 3-kinases-AKT, Rho, and downregulates peroxisome proliferator-activated receptor γ expression through the Smad pathway (21). In alveolar epithelial cells (AEC), TGF-β1 mediates epithelial-mesenchymal transition (EMT) leading to lung fibrosis (22). Previous studies have demonstrated that MK has a certain regulatory effect on TGF-β signaling pathway in various diseases such as asthma and pulmonary fibrosis (23-25). However, the effect of MK on TGF-β signaling pathway in in BPD remains largely unclear.

The present study investigated whether MK displayed an important role during BPD by regulating EMT, and further analyzed the molecular mechanism underlying its effects, with the aim of providing a theoretical basis for the use of MK as a therapeutic strategy for BPD.

Materials and methods

Establishment of the rat model of BPD. A total of ten pregnant Wistar rats (weight, 200-250 g; 10 weeks old) were purchased from the Animal Center of Nanjing Medical University. Animals were housed at 25±5˚C, with 50% humidity and 12 h light/dark cycles with *ad libitum* supply of food and water. A rat model of BPD was established according to a previous study (26). Briefly, the 15 pups of the pregnant Wistar rats were randomly divided into the following three groups (n=5): i) BPD model group; ii) BPD + MK group and; iii) control group. The model group rats were maintained in a sealed Plexiglass chamber with 85% oxygen for 14 days from the date of birth. The oxygen concentration in the chamber was continuously recorded using an analyzer with a strip-chart recorder (Servomex). The control group rats were maintained in room air. All animal procedures were performed according to the Institutional Animal Care and Use of Laboratory Animals guidelines by the National Institutes of Health. The present study was approved by the Animal Ethics Committee of The First Affiliated Hospital of Bengbu Medical College.

Treatment of the rat model of BPD. The BPD + MK group were administered with an intraperitoneal injection of 10 mg/kg MK (Sigma-Aldrich; Merck KGaA) every other day from day 2 to day 14 post-hyperoxia induction (27). The control and BPD model groups were administered with an intraperitoneal injection of the same amount of saline (0.9% NaCl). All efforts were made to alleviate the pain of the rats throughout the experiment. In addition, all experiments were stopped when the rats lost >15% of their body weight. The health and behavior of all rats were monitored every 2 days, and no rats died prior to the end of the experiment. Rats were anaesthetized by pentobarbital intraperitoneal injection (40 mg/kg) and subsequently sacrificed via cervical dislocation (rats without a heartbeat and not breathing were confirmed as dead) at 14 days after hyperoxia induction, for further experiments.

Lung sample collection and lung histology. Tissue samples from the middle lobe of the right lungs in the rats were aerated with PBS under a pressure of 18 cm H_2O. Subsequently, tissue samples were fixed in 4% paraformaldehyde (Beyotime Institute of Biotechnology) at 4˚C overnight. The lung tissues were embedded in paraffin and cut into 4-μm thick sections, following which they were rehydrated using an alcohol gradient (absolute ethanol for 5 min; 90% ethanol for 2 min; 80% ethanol for 2 min; 70% ethanol for 2 min; distilled water for 2 min), and stained with hematoxylin and eosin at room temperature for 10 min. The mean linear intercept (MLI), which represents the average alveolar diameter, was measured under a fluorescent microscope at x200 magnification. The number of alveoli transected by the line from the center of the most peripheral bronchiole to the pleura, which is defined as the radial alveolar count (RAC), was calculated to evaluate the stage of lung development. In addition, the right upper lung lobes of the rats were collected and weighed to determine the lung weight/body weight (LW/BW) ratio.

Type II alveolar epithelial (AEC II) cell isolation and culture. AEC II cells were isolated from three rat pups from the control group within 24 h of birth (28). Briefly, the lungs of newborn rat pups were collected and digested with 0.25% Trypsin-EDTA (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C. Subsequently, the lungs were digested with 0.1% collagenase I (Gibco; Thermo Fisher Scientific, Inc.) and filtered through a cell strainer (70 μm). The isolated cells were resuspended and purified by centrifugation (1,000 x g, 4˚C, 5 min). Cells were incubated in minimum essential medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. At passage 3, the purity and viability of AEC II cells was determined according to a previous study (28,29), and cells with 90% purity and >95% viability were used for subsequent experiments.

Cells were randomly divided into the following two groups: Hyperoxia-induced group (HOX group; cells cultured at 37˚C for 48 h in an 85% O_2-5% CO_2 incubator) and the normoxic control group (NOX group; cells cultured at 37˚C for 48 h in a 21% O_2-5% CO_2 incubator). The NOX group was further divided into three subgroups: i) Control; ii) transforming growth factor (TGF)-β1 (Sigma-Aldrich; Merck KGaA); and iii) TGF-β1 + MK (10 μM). Cells in the TGF-β1 group were cultured with TGF-β1 (3 ng/ml) at 37˚C for 48 h to induce EMT. Cells in the TGF-β1 + MK group were cultured with TGF-β1 (3 ng/ml) at 37˚C for 48 h to induce EMT and then treated with 10 μM MK at 37˚C for 24 h. The HOX group was further divided into five subgroups: i) Control; ii) hyperoxia; iii) hyperoxia + TGF-β1 antibody; iv) hyperoxia + MK; and v) hyperoxia + TGF-β1 antibody + MK. Cells in the control group did not undergo any treatment.

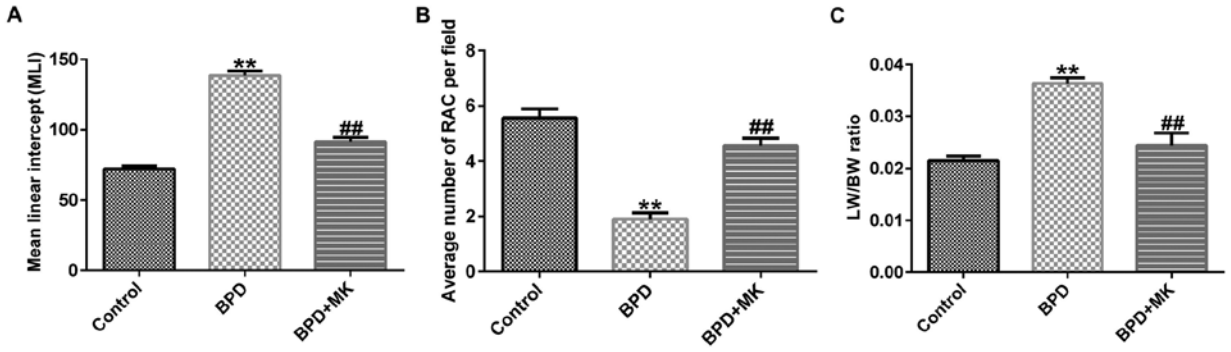

Figure 1. Effects of MK on alveolarization and lung injury. A rat model of BPD was established. Rats in the BPD + MK group received an intraperitoneal injection of 10 mg/kg MK every other day from day 2 to 14 post-birth. Rats in the control and BPD model groups received an intraperitoneal injection of saline solution. At 14 days post-treatment, the (A) MLI, (B) RAC and (C) LW/BW ratio of each rat was determined. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. BPD. BPD, bronchopulmonary dysplasia; MLI, mean linear intercept; RAC, radial alveolar count; LW/BW, lung weight/body weight; MK, montelukast.

Western blotting. The protein expression levels of surfactant protein C (SP-C), E-cadherin, N-cadherin, Vimentin, collagen I (Col I), matrix metallopeptidase (MMP)1/3, TGF-β1 and Smad3 in the tissue samples and cells were determined by western blotting. Briefly, total protein was extracted using a total protein extraction kit (Beyotime Institute of Biotechnology), and quantified using the Bicinchoninic Acid Protein kit (Pierce; Thermo Fisher Scientific, Inc.). Proteins (40 μg per lane) were loaded on a 10% gel, resolved via SDS-PAGE under reduced conditions, and subsequently transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk and PBS with 0.5% Tween-20 (PBST) solution at room temperature for 1 h. Subsequently, the membranes were incubated at 4˚C overnight with primary antibodies targeted against: SP-C (1:1,000; cat. no. ab211326; Abcam), E-cadherin (1:1,000; cat. no. ab233766; Abcam), N-cadherin (1:1,000; cat. no. ab76011; Abcam), Vimentin (1:1,000; cat. no. ab137321; Abcam), Col I (1:1,000; cat. no. ab34710; Abcam), MMP-1 (1:1,000; cat. no. ab137332; Abcam), MMP-3 (1:1,000; cat. no. ab52915; Abcam), TGF-β1 (1:1,000; cat. no. ab92486; Abcam), Smad3 (1:1,000; cat. no. ab40854; Abcam) and GAPDH (1:1,000; cat. no. ab181602; Abcam). Following primary incubation, the membrane was washed three times with PBST and incubated with a horseradish peroxidase-conjugated secondary antibody (1:1,000; cat no. ab7090; Abcam) for 1 h. Protein bands were visualized by enhanced chemical luminescence (Beyotime Institute of Biotechnology), according to the manufacturer's protocol. Protein expression levels were semi-quantified using ImageJ software version 1.46 (National Institutes of Health) with GAPDH as the loading control.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from the tissues and cells using an RNA Isolation kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and reverse transcribed into first strand cDNA using the cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The temperature protocol for the reverse transcription reaction were as following: annealing at 25˚C for 5 min, cDNA synthesis at 42˚C for 60 min and termination at 80˚C for 2 min. Subsequently, mRNA expression levels were assessed by qPCR using a Prism 7000 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd.), according to the manufacturer's protocol. The primer pairs used for qPCR were purchased from Sangon Biotech Co., Ltd. The following primer sequences were used: SP-C forward, 5'-TTGGTCCTTCACCTCTGTCC-3' and reverse, 5'-CTCCCACAATCACCACGAC-3'; Vimentin forward, 5'-GACGCCATCAACACCGAGTT-3' and reverse, 5'-CTTTGTCGTTGGTTAGCTGGT-3'; E-cadherin forward, 5'-CGAGAGCTACACGTTCACGG-3' and reverse, 5'-GGGTGTCGAGGGAAAAATAGG-3'; N-cadherin forward, 5'-TTTGATGGAGGTCTCCTAACA CC-3' and reverse, 5'-ACGTTTAACACGTTGGAAATGTG-3'; GAPDH forward, 5'-CTTTGGTATCGTGGAAGGACTC-3' and reverse, 5'-GTAGAGGCAGGGATGATGTTCT-3'. The following thermocycling conditions were used for qPCR: 5 min at 95˚C; followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. mRNA expression levels (SP-C, E-cadherin, N-cadherin, Vimentin) were quantified using the $2^{-\Delta\Delta Cq}$ method (30) and normalized to the internal reference gene GAPDH.

Statistical analysis. Data are presented as the mean ± standard deviation of at least three independent experiments. One-way ANOVA followed by Tukey's post hoc test was used for comparisons between multiple groups. Statistical analyses were performed using SPSS software (version 18; SPSS, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of MK on alveolarization and lung injury in a rat model of BPD. To assess the effects of MK on lung function, the MLI, RAC and LW/BW ratio of the lungs were assessed in the different groups. The MLI of the BPD model group was longer compared with the control group, and MK treatment significantly decreased the MLI in the BPD model group (Fig. 1A). Similarly, MK markedly significantly the RAC and decreased the LW/BW ratio in the BPD model group (Fig. 1B and C).

Effects of MK on EMT in a hyperoxia-induced rat model of BPD. The lung tissues of the rats were isolated at day 14

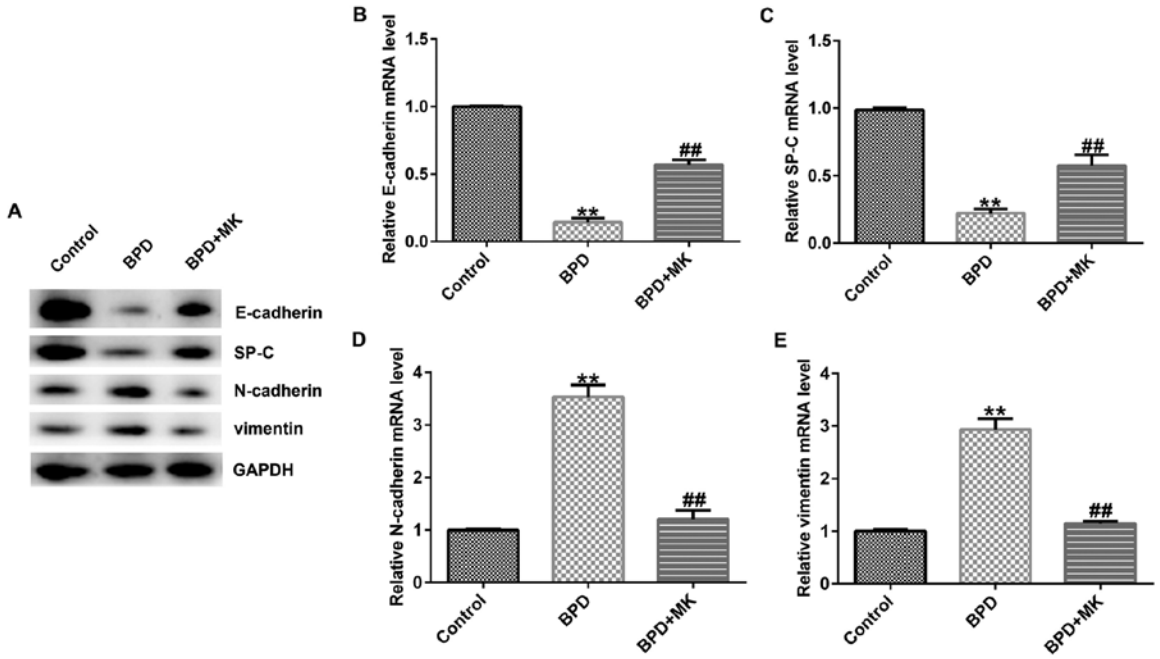

Figure 2. Effects of MK on epithelial-mesenchymal transition in a hyperoxia-induced rat model of BPD. Lung tissues were isolated at day 14 post-hyperoxia induction and were subsequently assessed. (A) The protein expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in lung tissues were detected by western blotting. (B-E) The mRNA expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in lung tissues were measured by reverse transcription-quantitative PCR. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. BPD. BPD, bronchopulmonary dysplasia; SP-C, surfactant protein C; MK, montelukast.

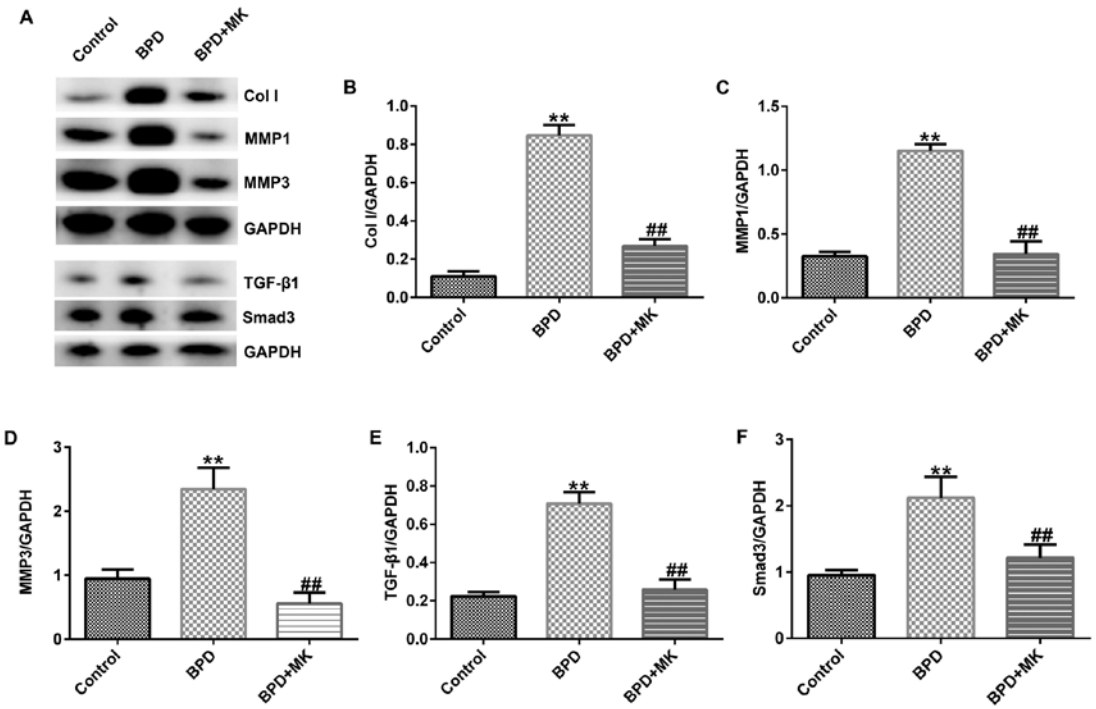

Figure 3. Effects of MK on the TGF-β signaling pathway in a hyperoxia-induced rat model of BPD. The protein expression levels of Col I, MMP1, MMP3, TGF-β1 and Smad3 were (A) measured by western blotting and (B-F) semi-quantified using ImageJ software. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. BPD. TGF-β, transforming growth factor-β; BPD, bronchopulmonary dysplasia; Col I, collagen I; MMP, matrix metallopeptidase; MK, montelukast.

post-hyperoxia induction, and the protein and mRNA expression levels of epithelial cell markers E-cadherin and SP-C, as well as mesenchymal cell markers N-cadherin and Vimentin, were measured by western blotting and RT-qPCR, respectively. The protein expression levels of E-cadherin and SP-C in the BPD model group were markedly decreased, whereas the protein expression levels of N-cadherin and Vimentin were increased compared with the control group (Fig. 2A). The mRNA expression levels of E-cadherin and SP-C in the BPD model group were significantly decreased,

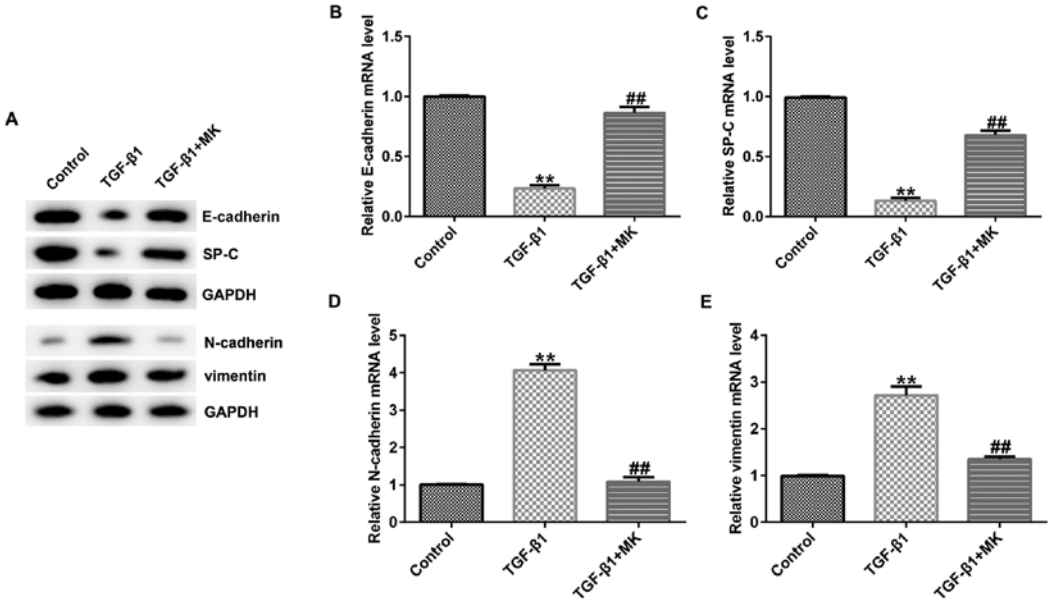

Figure 4. Effects of MK on epithelial-mesenchymal transition in TGF-β1-induced AEC II cells derived from normal rats. (A) Western blotting was performed to detect the protein expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in AEC II cells. (B-E) Reverse transcription-quantitative PCR was performed to detect the mRNA expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in AEC II cells. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. TGF-β1. TGF-β1, transforming growth factor-β1; AEC II, Type II alveolar epithelial; SP-C, surfactant protein C; MK, montelukast.

whereas the mRNA expression levels of N-cadherin and Vimentin were significantly increased compared with the control group (Fig. 2B-E). MK treatment notably inhibited BPD-induced effects on protein and mRNA expression levels (Fig. 2A-E).

Effects of MK on the TGF-β signaling pathway in a hyperoxia-induced rat model of BPD. In addition, western blotting was performed to measure the relative expression of TGF-β signaling pathway-related proteins Col I, MMP1/3, TGF-β1 and Smad3. Compared with the control group, the expression levels of Col I, MMP1 and MMP3 in the BPD model group were significantly increased, and MK treatment significantly inhibited these effects (Fig. 3A-D). Similarly, the expression levels of TGF-β1 and Smad3 in the BPD model group were increased compared with the control group, which were significantly decreased by treatment with MK (Fig. 3A, E and F).

Effects of MK on TGF-β1-induced EMT in AEC II cells derived from normal rats. AEC II cells were isolated from the normal rats and incubated with TGF-β1 to induce EMT. The protein and mRNA expression levels of E-cadherin, SP-C, N-cadherin and Vimentin were measured by western blotting and RT-qPCR. Compared with the control group, the protein expression levels of E-cadherin and SP-C in the TGF-β1 group were markedly decreased, whereas the protein expression levels of N-cadherin and Vimentin were notably increased, and these alterations were inhibited by treatment with MK (Fig. 4A). Compared with the control group, the mRNA expression levels of E-cadherin and SP-C in the TGF-β1 group were significantly decreased, whereas the mRNA expression levels of N-cadherin and Vimentin were significantly increased, and these alterations were inhibited by treatment with MK (Fig. 4B-E).

Effects of MK on the TGF-β signaling pathway in AEC II cells derived from normal rats. Furthermore, western blotting was performed to detect the protein expression levels of TGF-β signal pathway-related proteins Col I, MMP1/3, TGF-β1 and Smad3. The results indicated that the expression levels of Col I, MMP1 and MMP3 in the TGF-β1 group were significantly increased compared with the control group, which were significantly decreased by MK treatment (Fig. 5A-D). The expression levels of TGF-β1 and Smad3 in the TGF-β1 group were also increased compared with the control group, which were significantly decreased by treatment with MK (Fig. 5A, E and F).

Effects of MK on EMT in hyperoxia-induced AEC II cells. To further explore the effect of MK on EMT in AEC II cells derived from a hyperoxia-induced rat model of BPD, the effects of MK on hyperoxia-induced AEC II cells were investigated. AEC II cells were divided into five groups (hyperoxia group, hyperoxia + TGF-β1 antibody group, hyperoxia + MK group, hyperoxia + TGF-β1 antibody + MK group, and control group), and the protein and mRNA expression levels of E-cadherin, SP-C, N-cadherin and Vimentin were detected by western blotting and RT-qPCR, respectively. The results suggested that, compared with the control group, the protein expression levels of E-cadherin and SP-C in the hyperoxia group were reduced, and the protein expression levels of N-cadherin and Vimentin were notably increased (Fig. 6A). Compared with the control group, the mRNA expression levels of E-cadherin and SP-C in the hyperoxia group were significantly reduced, and the mRNA expression levels of N-cadherin and Vimentin were significantly increased (Fig. 6B-E). Furthermore, hyperoxia-induced alterations to protein and mRNA expression were inhibited by treatment with MK or TGF-β1 antibody, with the TGF-β1 antibody + MK group displaying the most effective inhibition (Fig. 6A-E).

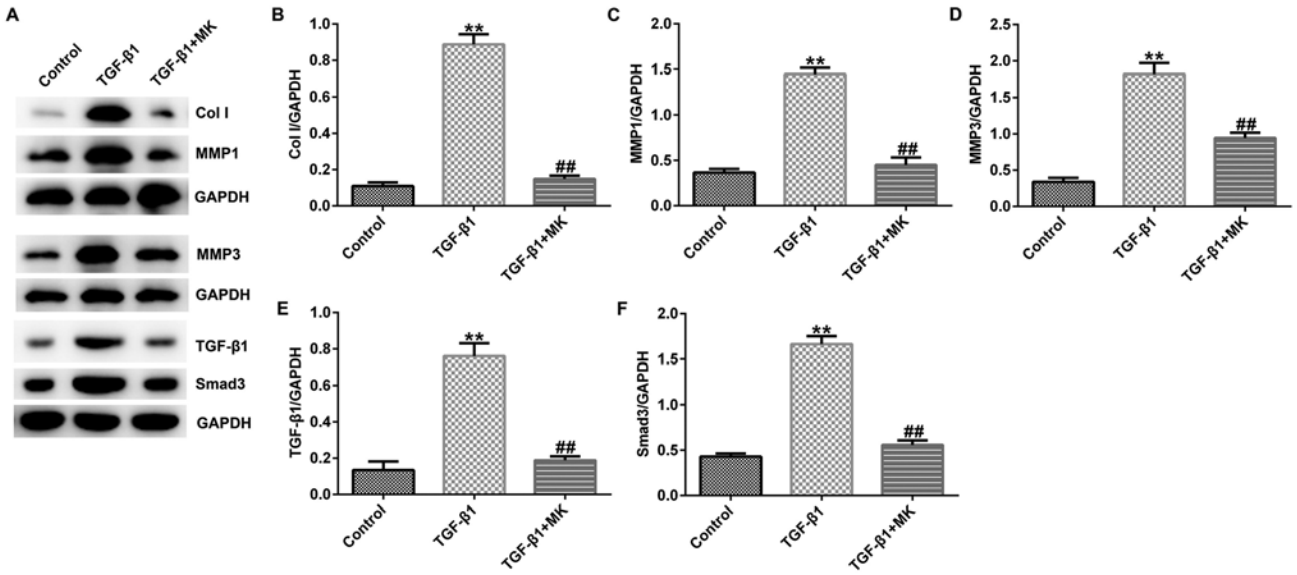

Figure 5. Effects of MK on the TGF-β signaling pathway in TGF-β1-induced AEC II cells derived from normal rats. The protein expression levels of Col I, MMP1, MMP3, TGF-β1 and Smad3 in AEC II cells were (A) determined by western blotting and (B-F) semi-quantified using ImageJ software. **P<0.01 vs. control; $^{\#\#}$P<0.01 vs. TGF-β1. TGF-β, transforming growth factor-β; AEC II, type II alveolar epithelial; Col I, collagen I; MMP, matrix metallopeptidase; MK, montelukast.

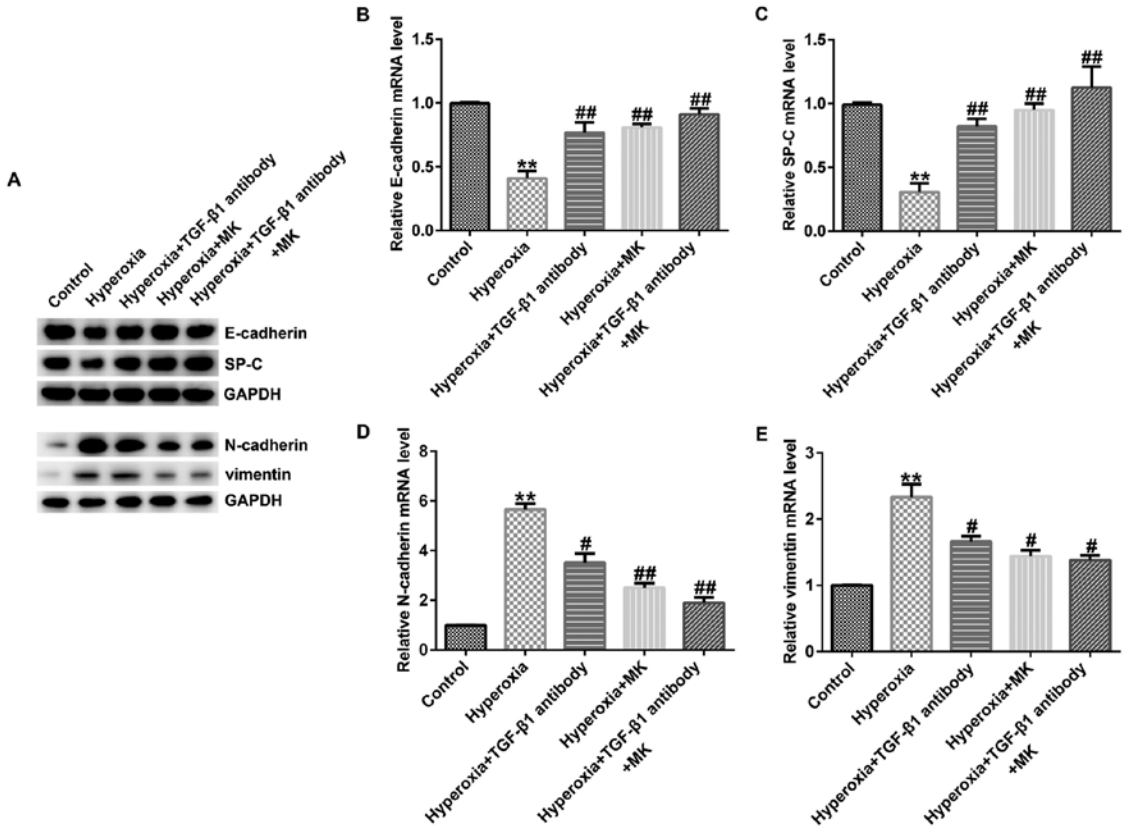

Figure 6. Effects of MK on epithelial-mesenchymal transition in hyperoxia-induced AEC II cells. The effect of MK on hyperoxia-induced AEC II cells was determined. Cells were divided into five groups: i) Hyperoxia group; ii) hyperoxia + TGF-β1 antibody group; iii) hyperoxia + MK group; iv) hyperoxia + TGF-β1 antibody + MK group; and v) control group. (A) Western blotting was performed to measure the protein expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in AEC II cells. (B-E) Reverse transcription-quantitative PCR was performed to measure the mRNA expression levels of E-cadherin, SP-C, N-cadherin and Vimentin in AEC II cells. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. hyperoxia. AEC II, type II alveolar epithelial; MK, montelukast; TGF-β1, transforming growth factor-β1; SP-C, surfactant protein C.

Effects of MK on the TGF-β signaling pathway in hyperoxia-induced AEC II cells. The hyperoxia group displayed a significant increase in the expression levels of Col I, MMP1 and MMP3 compared with the control

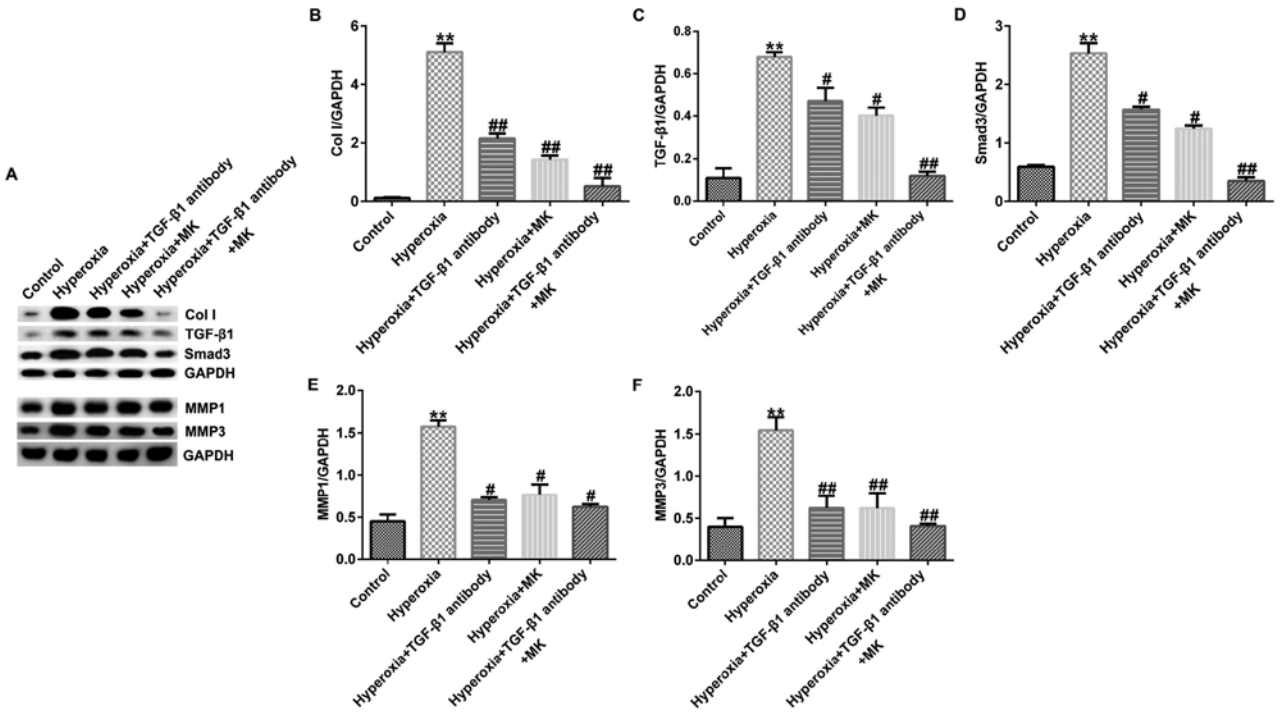

Figure 7. Effects of MK on the TGF-β signaling pathway in TGF-β1-induced AEC II cells derived from a rat model of BPD. The protein expression levels of Col I, MMP1, MMP3, TGF-β1 and Smad3 in AEC II cells derived from a rat model of BPD were (A) determined by western blotting and (B-F) semi-quantified using ImageJ software. **P<0.01 vs. control; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. hyperoxia. TGF-β, transforming growth factor-β; AEC II, type II alveolar epithelial; BPD, bronchopulmonary dysplasia; Col I, collagen I; MMP, matrix metallopeptidase; MK, montelukast.

group, which was notably inhibited by treatment with MK or TGF-β1 antibody. The combined treatment of MK and TGF-β1 was more effective compared with either treatment alone (Fig. 7A, B and F). Furthermore, compared with the control group, the expression levels of TGF-β1 and Smad3 in the hyperoxia group were markedly increased, and hyperoxia-induced effects were suppressed by treatment with MK or TGF-β1 (Fig. 7A, C and D).

Discussion

BPD is caused by immature lung development in premature infants, resulting in a cascade secondary inflammatory response induced by high oxygen injury, volume injury, barotrauma, infection and open arterial catheters (31,32). A large number of inflammatory cells infiltrate the lungs and release a high number of inflammatory mediators, which lead to further lung injury, and eventually result in pulmonary fibrosis and BPD (33,34). Therefore, the current treatment strategies for BPD primarily focus on reducing the secondary inflammatory response and lung injury induced by abnormal repair.

There have been several related studies on the effect of MK on BPD (14,15,27). Besides, Kim *et al* (35) have reported the efficacy and safety of MK sodium in the prevention of bronchopulmonary dysplasia. However, the effect and mechanism of MK on BPD still need to be further explored. In the present study, a hyperoxia-induced rat model of BPD was established to investigate the effects of MK during BPD. Rats were treated with 10 mg/kg MK and lung function was evaluated at day 14 post-hyperoxia induction. The MK group displayed a significantly lower MLI and LW/BW ratio, and an increased RAC compared with the control group, suggesting that MK displayed a protective effect against lung injury in the rat model of BPD. However, this study did not include the histology data, which is a limitation of the present study.

Abnormal repair and pulmonary fibrosis following acute lung injury leads to BPD, EMT results in pulmonary fibrosis, disease progression and deterioration of BPD (36). Previous studies have reported that the TGF-β signaling pathway is aberrantly activated during the progression of EMT (37,38); therefore, it was hypothesized that MK may improve BPD by inhibiting the process of EMT. Western blotting and RT-qPCR were performed to detect alterations to the expression levels of EMT- and TGF-β signaling pathway-related genes. The results indicated that MK upregulated the expression levels of the epithelial-related genes SP-C and E-cadherin, and downregulated the expression levels of the interstitial-related genes Vimentin and N-cadherin in the lung tissues. In addition, MK decreased the expression of Col I, MMP, MMP3, TGF-β1 and Smad3. The results indicated that MK inhibited the process of EMT and the TGF-β signaling pathway in the hyperoxia-induced rat model of BPD.

AEC II cells are pulmonary epithelial progenitor cells, and AEC II cell injury is an important mechanism underlying BPD-related pulmonary epithelial injury (39). In the present study, AEC II cells were isolated from the lungs of normal rats and the rat model of BPD, and subsequently, the effects of MK on EMT and the TGF-β signaling pathway in AEC II cells were investigated. Similarly, the results indicated that MK inhibited the process of EMT and the TGF-β signaling pathway in AEC II cells.

In conclusion, the results suggested that MK improved BPD by mediating EMT in AEC II cells via inhibiting the TGF-β1/Smads signaling pathway. The present study suggested that MK displayed protective effects against BPD, which indicated that MK might serve as a potential therapeutic agent for BPD.

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Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XC and WP contributed to study design, data collection, statistical analysis, data interpretation and manuscript preparation. ZZ contributed to data collection and statistical analysis. RZ and JX contributed to data collection, statistical analysis and manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were performed according to the Institutional Animal Care and Use of Laboratory Animals guidelines by the National Institutes of Health. The present study was approved by the Animal Ethics Committee of The First Affiliated Hospital of Bengbu Medical College.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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