ผลของสมุนไพรฟ้าทะลายโจรต่อการขนส่งไอออน ในเซลล์เยื่อบุผิวทางเดินหายใจมนุษย์

Effect of *Andrographis paniculata* on Ion Transport in Human Respiratory Epithelium

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บทคัดย่อ

ฟ้าทะลายโจร (*Andrographis paniculata*) เป็นสมุนไพรที่ใช้กันอย่างแพร่หลายมานานหลายศตวรรษในประเทศจีน อินเดีย และประเทศในแถบเอเชียตะวันออกเฉียงใต้ โดยใช้สำหรับการรักษาโรคติดเชื้อทางระบบหายใจและบรรเทาอาการหวัด อย่างไรก็ตาม การศึกษาฤทธิ์ของฟ้าทะลายโจรที่เกี่ยวข้องกับการควบคุมการขนส่งไอออนในเซลล์เยื่อบุผิวทางเดินหายใจยังไม่เป็นที่ทราบแน่ชัด งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของฟ้าทะลายโจรต่อกลไกการขนส่งไอออนในเซลล์ H441 ซึ่งเป็นเซลล์เยื่อบุผิวทางเดินหายใจมนุษย์โดยใช้เทคนิค Ussing Chamber ในการคำนวณค่ากระแสลัดวงจรสมมูล ซึ่งแสดงถึงการขนส่งไอออนสุทธิที่เกิดขึ้นผ่านเซลล์เยื่อบุผิว จากการทดลองพบว่า ฟ้าทะลายโจรทำให้เกิดการเพิ่มขึ้นของค่าความต่างศักย์ไฟฟ้าระหว่างด้านบนและด้านล่างของเซลล์เยื่อบุผิวทางเดินหายใจและค่ากระแสลัดวงจรสมมูล ($p < 0.001$; $n = 5$) โดยผลการออกฤทธิ์ของฟ้าทะลายโจรจะหายไปเมื่อมีการยับยั้งการทำงานของเยื่อบุช่องโซเดียมด้วยอะมิโลไรด์ ซึ่งเป็นสารที่ขัดขวางการเคลื่อนที่ของโซเดียมไอออนเข้าสู่เซลล์อย่างแรง ($p < 0.001$; $n = 5$) การทดลองนี้ยังพบว่า การออกฤทธิ์ของฟ้าทะลายโจรไม่ผ่านการทำงานของเยื่อบุช่องคลอไรด์ ชนิด TMEM16A และ CFTR ซึ่งศึกษาโดยใช้สารยับยั้งการทำงานของเยื่อบุช่องคลอไรด์ ผลการศึกษาครั้งนี้สรุปได้ว่า ฟ้าทะลายโจรมีฤทธิ์ทำให้เซลล์ H441 มีการดูดซึมของโซเดียมไอออนผ่านทางเยื่อบุช่องโซเดียมเพิ่มขึ้น แต่ไม่ทำให้การคัดหลั่งของคลอไรด์เปลี่ยนแปลง

คำสำคัญ: ฟ้าทะลายโจร เซลล์เยื่อบุผิวทางเดินหายใจมนุษย์ การดูดซึมของโซเดียมไอออน

Abstract

Andrographis paniculata, also known as Fah Talai Jone in Thailand, is an herb traditionally used in China, India and Southeast Asia. It has been widely used for centuries, for the treatment of respiratory infection and common cold. However, the effect of *Andrographis paniculata* in regulating ion transport in respiratory epithelium is currently has not well understood. Thus, the present study was aimed to determine the effect of *Andrographis paniculata* on ion transport in human respiratory epithelium using NCI-H441 cell line using Ussing chamber technique. The equivalent short-circuit (I_{sc}), an indicator of ion transport across the epithelium was calculated. The results showed that *Andrographis*

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paniculata increased transepithelial potential difference and I_{sc} ($p < 0.001$; n =5). The effect of *Andrographis paniculata* was abolished when the cell monolayer was pre-treated with amiloride, a potent epithelial sodium channel (ENaC) blocker ($p < 0.001$; n = 5). Interestingly, the effect of *Andrographis paniculata* was not sensitive to TMEM16A and CFTR Cl^- channel blockers. Taken together these findings suggest that *Andrographis paniculata* increases Na^+ absorption via ENaC without any effect on Cl^- secretion in H441 cells.

Keywords: *Andrographis paniculata*, Human Respiratory Epithelium, Na^+ absorption

Introduction

Andrographis paniculata, commonly known as Fah Talai Jone (King of Bitters), is an herbal medicine that has been widely used in Chinese, Thai, and Ayurvedic medicine for treatment of asthma, respiratory tract infection [1], common cold [2], influenza and fever [3]. Pharmacological studies suggest anti-inflammatory [4-5], antipyretic [6], antiviral [7] and immunostimulatory [8] properties. Some clinical studies in uncomplicated common cold patients showed that the effectivity of *Andrographis paniculata* was much pronounced in controlling nasal secretion and nasal congestion [9]. However, the mechanism of this anti-secretory action remains unknown. Because airway surface fluid consists of mucous glycoprotein released from submucosal glands and water transported across airway epithelial cells, the latter being determined by transepithelial ion transport [10]. In particular, it is regulated by the balance between Na^+ absorption mediated by epithelial sodium channel (ENaC) and Cl^- secretion mediated by cystic fibrosis transmembrane conductance regulator (CFTR) and the calcium-activated Cl^- channel (CaCC) [11].

H441 cell is a respiratory epithelial cell line that has been used extensively as a suitable model for studying electrolyte and fluid transport in the respiratory epithelium. So far the effect of *Andrographis paniculata* in regulating ion transport in this cell type has not yet been well understood. The present study aimed to determine the effect of *Andrographis paniculata* on respiratory epithelium ion transport functions by measured bioelectric properties of H441 monolayer, which primarily secreates Cl^- and absorbs Na^+ [12], under short-circuit *in vitro* condition.

Materials and Methods

Cell culture: The human lung adenocarcinoma epithelial cell line, NCI-H441 cells (ATCC® HTB-174™), was a gift from Assoc. Prof. Dr. Anuwat Dinudom (University of Sydney, New South Well, Australia). H441 cells were grown in RPMI 1640 medium supplemented with 9 % fetal bovine serum, 2 mM L-glutamine, 5 µg/ml insulin, 5 µg/ml transferring, 5 ng/ml selenium and 100 U/ml penicillin and streptomycin. The cells were maintained in 75 cm^2 flasks and incubated in a humidified atmosphere of 5 % CO_2 at 37 °C until they reach 70 % confluence before plating on Snapwell (Corning, U.S.A). For Ussing chamber studies, the cells were plated on Snapwell at the density 250,000 cells/insert and grown in RPMI 1640 medium supplemented with 100 nM dexamethasone. After seeding for 24 hr, apical side of the cells were washed and 100 µl of fresh media added whereas the bottom compartment of the Snapwell were filled with 1.5 ml of fresh media and subsequently changed every 48 hr. Monolayers were incubated for 4-5 days until tight junctions were developed.

Ussing chamber study: Ussing chamber technique was used to determine the equivalent short-circuit current representing ion transport property of H441 cell monolayers grown on permeable supports (Snapwell). The Ussing chamber is separated into two compartments, apical and basolateral, divided by the monolayer. Each half of the chamber was filled with physiological bathing solution containing (in mM) NaCl (130), KCl (4), H-HEPES (5), Na^+-HEPES (5), $MgCl_2$ (1), $CaCl_2$ (1) and glucose (5); pH 7.4. The chamber was connected to a data acquisition system (PowerLab 4/30, ADInstruments, New South Wales, Australia) and a high impedance device to prevent current to be drawn from the circuit by equipment. Transepithelial potential difference (V_{te}) was monitored continuously using a Chart V 7 program with reference to the basolateral side of the epithelium. Transepithelial resistance (R_{te}) was determined by applying short (200 ms) current pulses (3 μA, period 6 s). The equivalent short-circuit current (I_{sc}) was calculated according to Ohm's Law (I = V/R). Short-circuit current was allowed to stabilize for approximately 10 min before performing the experiment.

After the H441 monolayers were allowed to equilibrate in the Using chamber for 10-20 min, *Andrographis paniculata* extract (Traditional Thai medicine Hospital, Prince of Songkla University, Songkhla, Thailand) dissolved in DMSO, at a concentration of 1, 10, 100 or 1000 μg/ml, was added to the apical bathing solution and the changes in V_{te} were recorded for at least 20 min. Pharmacological inhibitors were added to the apical side of the chamber 10 min prior to expose to the *Andrographis paniculata*. These include, at final concentration, 10 μM amiloride (Sigma, Missouri, U.S.A.), 20 μM CFTRinh-172 (Sigma, Missouri, U.S.A.) and 30 μM T16Ainh-A01 (Tocris Bioscience, Bristol, U.K.).

Statistical analysis: All data were expressed as mean ± standard error (S.E.M.) from at least 5 sets of experiments. Statistical difference was assessed using the one-way analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc test and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

The basal transepithelial potential difference (V_{te}) and transepithelial resistance (R_{te}) of H441 cells monolayers were 23.19 ± 1.23 mV (n = 25) and 1649.80 ± 81.94 $\Omega.cm^2$ (n = 25), respectively. These corresponded to an equivalent short-circuit current (I_{sc}) of 14.96 ± 1.02 $\mu A/cm^2$ (n = 25), calculated with Ohm's law. To determine the effect of *Andrographis paniculata* on ion transport in H441 cells, as illustrated in the representative tracings (Figure 1), exposing the apical membrane of H441 cell monolayers with *Andrographis paniculata* (AP) 1000 μg/ml caused a 7.33 ± 1.03 mV ($n = 5$, $p < 0.001$) increase in V_{te} (apical side becomes more negative, Figures 1E and 2A), which reached a plateau level within 10 min. This correspond to 3.61 ± 0.57 $\mu A/cm^2$ ($n = 5$, $p < 0.001$) increase in I_{sc} (Figure 2B). Whereas AP 100 μg/ml caused an increase in V_{te} (4.54 ± 0.89 mV, $n = 5$, $p < 0.001$; Figures 1D and 2A) but has no effect on I_{sc} of H441 cells (0.20 ± 0.55 $\mu A/cm^2$, n = 5; Figure 2B). Giving that DMSO showed no effect on the V_{te} (Figures 1A and 2A) and I_{sc} (Figure 2B), indicating that the changes in V_{te} and I_{sc} observed in this study were not an effect of solvent.

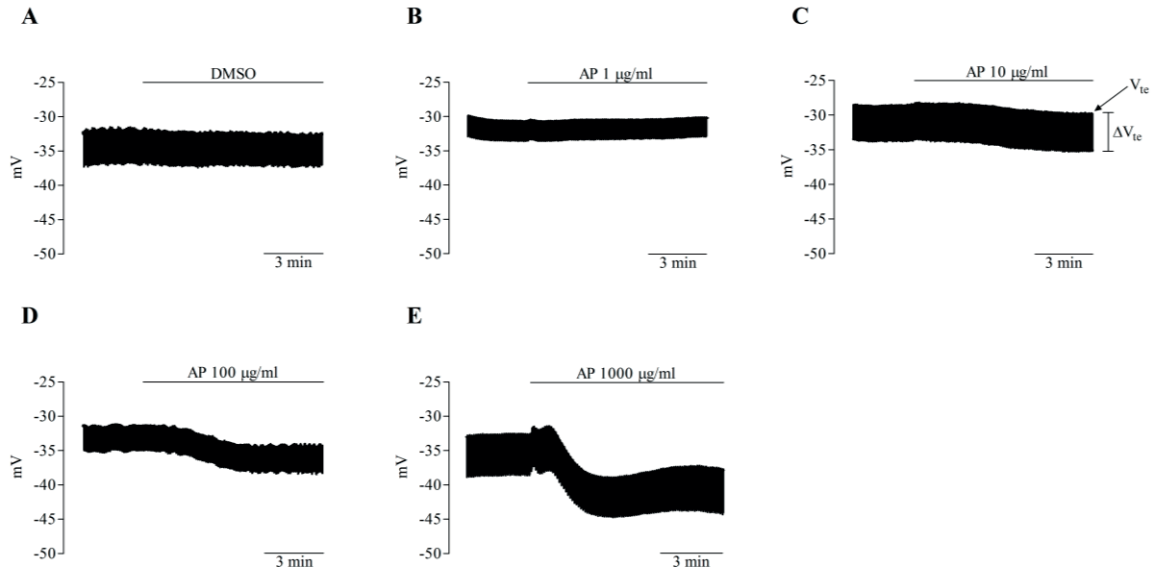

Figure 1 The effect of *Andrographis paniculata* (AP) on transepithelial voltage (V_{te}) in H441 cells. Representative recordings of V_{te} in H441 cells in response to (A) DMSO, (B) AP 1 µg/ml, (C) AP 10 µg/ml, (D) AP 100 µg/ml, and (E) AP 1000 µg/ml were added into apical bathing solution 10-20 min after start recording. V_{te} indicates transepithelial potential, ΔV_{te} indicates voltage changes in response to 3 µA injecting current and solid bar indicates the duration in which AP present in the apical bath solution.

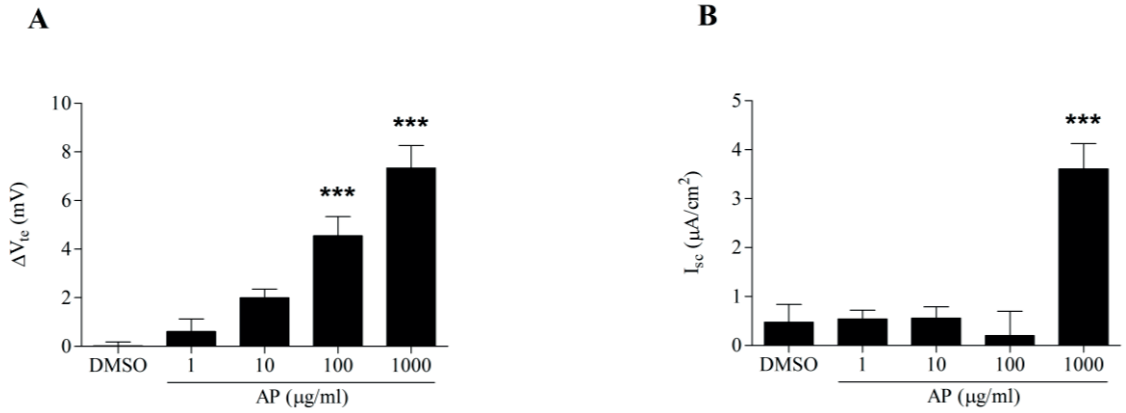

Figure 2 The effect of *Andrographis paniculata* (AP) on voltage changes (ΔV_{te}) and equivalent short-circuit current (I_{sc}) in H441 cells. The apical side of H441 monolayers was added with DMSO, AP 1, 10, 100 or 1000 µg/ml. Values are mean ± SEM from at least 5 sets of experiments. *** indicates $p < 0.001$ compared to negative control, DMSO (one-way ANOVA with Student-Newman-Keuls post-hoc test).

An increase in I_{sc} subsequent to *Andrographis paniculata* treatment may be due to either increasing cation absorption or anion secretion. The major route for cation absorption in respiratory cells is via the amiloride-sensitive pathway [13]. To determine the amiloride-sensitive component of the *Andrographis paniculata*–induced I_{sc} response, the H441 monolayers were pre-treated with 10 µM amiloride, a potent epithelial sodium channel (ENaC) blocker, by

added into the apical bath solution to eliminate the contribution of ENaC on I_{sc}. Under this condition, 1000 µg/ml AP-induce V_{te} and I_{sc} was 0.82 ± 0.34 mV (n = 5, p < 0.001; Figures 3B and 4A) and 0.46 ± 0.16 µA/cm^2 (n = 5, p < 0.001; Figure 4B), respectively. These results suggest a strong possibility that the mechanism by which *Andrographis paniculata* increase I_{sc} in H441 cells is mediated via ENaC. This finding is different with a previous report [14] that bisandrographolide, an active compound from *Andrographis paniculata* extracts, activates TRPV4 channel, nonselective cation channels, a Ca^{2+}-permeable in HEK293T cells.

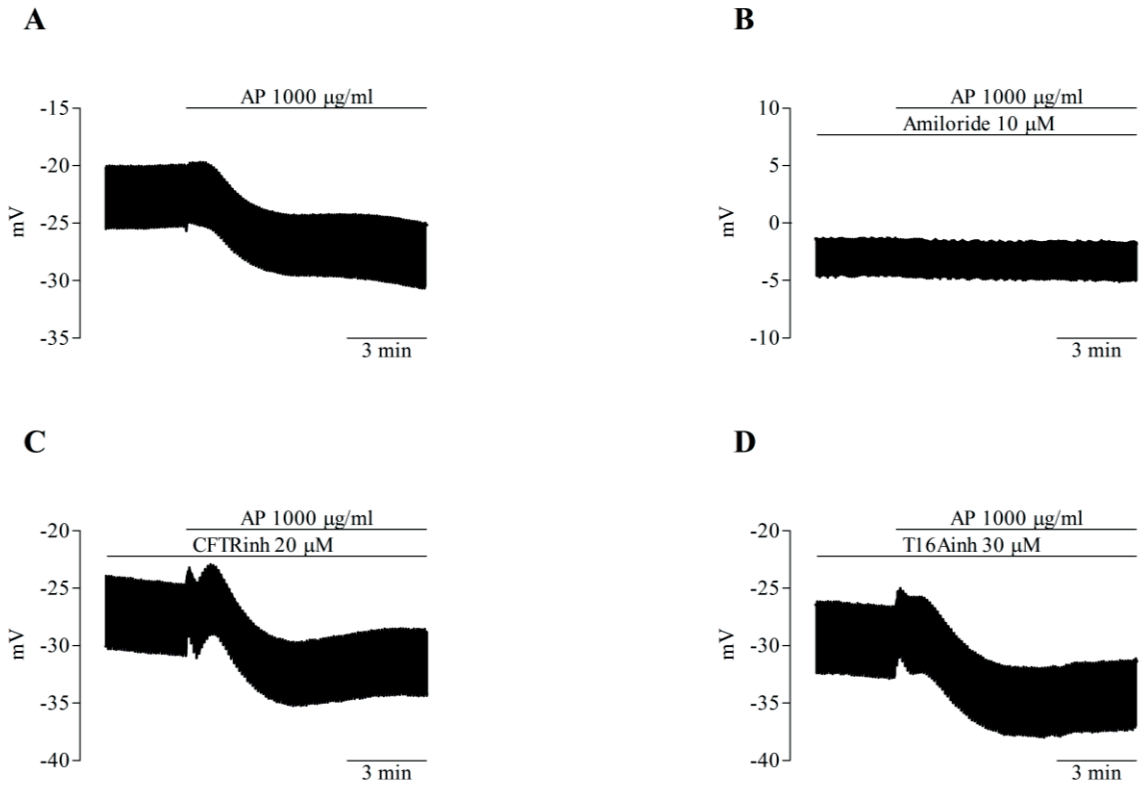

Figure 3 Representative recordings of transepithelial potential difference (V_{te}) in H441 cells. When the cell monolayers were untreated (A), pre-treated with 10 µM amiloride (B), 20 µM CFTRinh-172 (C) or 30 µM T16Ainh-A01 (D) 10 min before treating with *Andrographis paniculata* (AP, 1000 µg/ml).

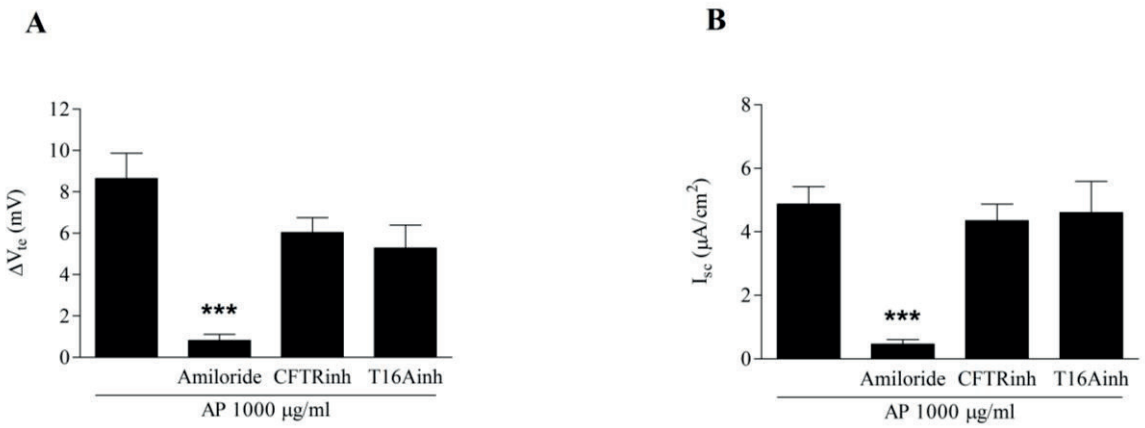

Figure 4 Effect of amiloride, CFTRinh-172, and T16Ainh-A01 on voltage changes (ΔV_{te}) and equivalent short-circuit current (I_{sc}) in H441 cells in response to AP 1000 μg/ml. The apical side of H441 monolayers pre-treated without and with 10 μM amiloride, 20 μM CFTRinh-172 or 30 μM T16Ainh-A01 10 min before the presence of AP (1000 μg/ml). Values are mean ± SEM from at least 5 sets of experiments. *** indicates $p < 0.001$ compared to cells treated with AP (1000 μg/ml) alone (one-way ANOVA with Student-Newman-Keuls post-hoc test).

Next, the effect of *Andrographis paniculata* on anion transport was determined by pre-treated H441 monolayers with 20 μM CFTRinh-172, a cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel inhibitor before the AP was introduced. Under this condition, V_{te} was increased by 6.03 ± 0.79 mV (n = 6; Figures 3C and 4A), corresponding to a 4.34 ± 0.58 μA/cm^2 (n = 6; Figure 4B) increasing in I_{sc}. These values were equivalent to those of AP-treated group. In a separate experiment, the cell monolayers were treated with 30 μM T16Ainh-A01, an inhibitor of TMEM16A channel, a calcium-activated chloride channel (CaCC). Under this condition, the inhibitor did not show any effect on AP-induced V_{te} (5.28 ± 1.22 mV, n = 6; Figures 3D and 4A) or I_{sc} (4.60 ± 1.07 μA/cm^2, n = 6; Figure 4B) when compare with the cells treated with AP alone. These data suggest that *Andrographis paniculata* has no any effect on the activity of both CFTR Cl^- channel or TMEM16A calcium-activated Cl^- channel.

Conclusion

This study was the first to determine physiological activity of *Andrographis paniculata* in H441 respiratory epithelial cells. The stimulatory effect of *Andrographis paniculata* on the Na^+ absorption across the respiratory epithelium could be beneficial in treating patients with respiratory infection such as common cold that associated with mucus and fluid accumulation in the airway. So far, the intracellular signaling pathway of *Andrographis paniculata* is still not well understood. More investigation to this signaling pathway is, therefore, required for a better understanding the action of *Andrographis paniculata* on airway diseases.

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