

Sanguinarine Decreases Cell Stiffness and Traction Force and Inhibits the Reactivity of Airway Smooth Muscle Cells in Culture

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Abstract: Airway hyperresponsiveness (AHR) is the cardinal character of asthma, which involves the biomechanical properties such as cell stiffness and traction force of airway smooth muscle cells (ASMCs). Therefore, these biomechanical properties comprise logical targets of therapy. β_2 -adrenergic agonist is currently the mainstream drug to target ASMCs in clinical practice for treating asthma. However, this drug is known for side effects such as desensitization and non-responsiveness in some patients. Therefore, it is desirable to search for new drug agents to be alternative of β_2 -adrenergic agonist. In this context, sanguinarine, a natural product derived from plants such as bloodroots, that has been reported to relax gut smooth muscle emerges as a potential candidate. So far, it is unknown whether sanguinarine can regulate the biomechanical properties of ASMCs and reactivity of ASMCs to irritants. Thus, we tested the hypothesis that sanguinarine reduce the contractile potentials of ASMCs in culture. To do so, the primary cultured rat ASMCs were first treated with different concentration of sanguinarine. Then, cell stiffness, traction force, fiber distribution, and calcium signaling of the ASMCs were evaluated by optical magnetic twisting cytometry, Fourier transform traction microscopy, atomic force microscopy, and Fluo-4/AM based fluorescence confocal scanning microscopy, respectively. The results indicated that sanguinarine (0.05 and 0.5 $\mu\text{mol/L}$) significantly decreased cell stiffness and traction force, inhibited reactivity of ASMCs to histamine, and disrupted the fiber structures in ASMCs in dose-dependent manner. These findings establish that sanguinarine can indeed change the biomechanical properties of ASMCs and may be used to treat AHR in asthma.

Keywords: Airway smooth muscle cells; sanguinarine; biomechanics; cell stiffness; cell traction force

1 Introduction

Airway hyper-responsiveness (AHR), the cardinal character of asthma, reflects that bronchial airways narrow too much and too easily [1,10], which leads to airway obstruction and restriction of asthmatic subjects. These processes are instigated by the shortening of airway smooth muscle cells (ASMCs) [4,20] after receiving contraction agonists, such as histamine (His). Therefore, ASMCs are the major effector cells of AHR [19,23], and thus are important targets for asthma therapy [6,17,36].

AHR is ultimately a mechanical process [12,29]. The direct cause of exaggerated airway narrowing is the increased maximum shortening velocity and contractility of ASMCs, which are essentially regulated by the biomechanical properties of ASMCs involving cell stiffness, cell traction force, cytoskeleton (CSK) organization, and excitation-contraction coupling signaling [20]. Studies using isolated bronchial rings and cultures of isolated ASMCs have shown that asthmatic ASMCs are

intrinsically different from non-asthmatic ones: they are both stiffer and hypercontractile [5,23,26]. Therefore, it is possible to decrease AHR by down regulating cell stiffness and contraction. Currently in clinical practice, β_2 -adrenergic agonists are commonly used to relax ASMCs for the purpose of bronchodilation. These drugs can control the asthma symptoms, but they often become tolerated and have some side effects. Most import is that these broncodilators have a very limited effect on attenuating AHR. Thus, it is urgent to identify new relaxation drug agents for ASMCs based on reducing AHR, particularly favoring those derived from natural materials such as plants.

One potential candidate is sanguinarine, a benzophenanthridine isoquinoline alkaloid derived from *Sanguinaria canadensis* and other poppy-fumaria species. Extensive previous studies have found that sanguinarine has various medical functions such as anti-inflammation, anti-oxidation, and anti-tumor. Interestingly, in a more recent study, Park and colleague [21] proposed a new approach to identify novel drugs according to the potential drug agent's effect on cell traction force, and in this way they screened the 1,120 drugs from Prestwick Chemical Library® that are already approved by the FDA or European Medicines Agency (EMA). Consequently, they found that sanguinarine blunts the contraction force of Schlemm's canal endothelial cells. Additionally, it has been shown that sanguinarine can cause either relaxation in blood vascular smooth muscle [15], or contraction in gut and uterine smooth muscle of rats [30,22]. However, it is unknown whether sanguinarine affects the biomechanical properties of ASMCs.

Therefore, in this study, we evaluated the effects of sanguinarine on the ASMCs' biomechanical properties including cell stiffness, traction force and reactivity to histamine (His) stimulation, which are important contributors to the excitation-contraction coupling and hyperresponsiveness of ASMCs in asthma. Our results indicated that sanguinarine could indeed decrease the stiffness and traction force, inhibit the reactivity to histamine, and disrupt the fiber structures in ASMCs. Considering that sanguinarine exhibits effects of both muscle relaxation and anti-inflammation, it is possible that sanguinarine is more advantageous than conventional β_2 -adrenergic agonist in treatment of AHR in asthma.

2. Materials and Methods

2.1 Materials

Anti-rat α -smooth muscle actin primary antibody, anti-rabbit IgG-FITC secondary antibody, Fluo-4/AM and collagen type I were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) penicillin, streptomycin and trypsin were purchased from Gibco (Grand Island, NY). Cell culture flasks and plates were purchased from Corning Incorporated (Corning, NY). Sanguinarine, transferrin and bovine insulin were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other reagents were obtained from Fisher Scientific (Newark, DE), unless noted otherwise.

2.2 Experimental Animals

Sprague Dawley (SD) rats (weight, 180-220 g; age, 6-8 weeks) were purchased from Cavens Lab Animal Co. Ltd. (Changzhou, China), an authorized supplier of experimental animals for use in medical research. The animals were maintained in a specific pathogen-free environment at room temperature (~25°C), relatively humidity of 40-60%, 12 h light/dark cycle and free access to food and water. All animal experiments were performed according to Institutional Guidelines for Animal Care and Use Committee of the Changzhou University (Changzhou, China). Adequate measures were taken to minimize the suffering of the experimental animals.

2.3 Isolation and in Vitro Culture of the Rat ASMCs

Primary ASMCs were isolated from the SD rats (approved by the Committee of Changzhou University on Studies Ethics) and cultured *in vitro* according to the method described previously [33] Briefly, SD rats were *i.p.* injected with pentobarbital sodium (60 mg/kg). The isolated tracheas were cleaned of connective tissues, cut longitudinally through the cartilage, and enzymatically dissociated with

Hanks' balanced salt solution (HBSS), consisting of (in mM): 5 KCl, 0.3 KH_2PO_4 , 138 NaCl, 4 NaHCO_3 , 0.3 Na_2HPO_4 , and 1.0 glucose, and containing 0.25% trypsin-0.02% EDTA solution, for 20 min at 37°C. Dissociated cells in suspension were centrifuged and suspended in DMEM supplemented with 10% FBS and antibiotics (100 U/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin). Cells were plated on culture flasks and grew until confluence at 37°C in humidified air containing 5% CO_2 .

A monoclonal antibody that recognizes only α -smooth muscle actin was used to identify ASMCs. Cells at passage of 3-10 were used for experiments. These cells maintain smooth muscle cell morphology and physiological response to agonists.

2.4 Assessment of Single Cell Morphology and Mechanical Properties of ASMCs

According to the method described previously [18,27], single cell morphology and mechanical properties of ASMCs were characterized at nanometer scale by using a Nanowizard II atomic force microscope (AFM, JPK Instruments AG, Berlin, Germany) mounted on an Olympus IX 81 inverted light microscope. After ASMCs were seeded on Petri dishes for 24 h, the medium was changed with HEPES-buffered serum-free culture medium containing different concentrations of sanguinarine (0.005 μM , 0.05 μM , or 0.5 μM). After treatment for 12 h, the dishes were mounted in a Petri dish heater (JPK Instruments) set to 37°C, cell surface morphology and stiffness were measured with AFM.

More specifically, single-cell imaging was used in contact mode with a cantilever with a spring constant of 10 mN/m (MLCT, Bruker AFM, Proes, Camarillo, CA) to obtain topographic images of the cell, and each cell was scanned for three times. The silicon nitride tips were irradiated with ultraviolet for 15 min to remove any organic contaminants prior to use. All measurements were recorded with a set point of 1 nN normal force and tip velocities of 2 $\mu\text{m s}^{-1}$ [22]. All images were analyzed by the instrument-equipped software to gain information of the topography. Cell height and surface roughness were given as means averaged across the analytical area.

After imaging the whole cell topology, we selected local regions of the cell surface for force-distance measurements using the force spectrum mode of AFM. Force curves were obtained by standard retraction. All force-distance experiments were performed at the same loading rate. The stiffness of the single cell was determined from the slope of the linear portion of the force indentation curve, which was similar to the deflection of the cantilever. Adhesion forces were induced by the interactions between the tip and the cell membrane. All data were mean \pm standard deviation (SD) of more than 100 force-distance curves taken from 5 to 10 different cells.

2.5 Assessment of Collective Cell Stiffness in Monolayer of ASMCs

Collective cell stiffness in monolayer of ASMCs was measured by optical magnetic twisting cytometry (OMTC) as described previously [2]. Briefly, RGD-coated ferrimagnetic microbeads (4.5 μm diameter) were added to ASMCs plated for 4-6 h on type I collagen-coated rigid dishes (96-well plate, Immulon II) for 20 min. Unbound beads were washed away with serum free medium, and then the microbeads were magnetized horizontally (parallel to the surface on which cells were plated) with a brief 1,000-Gauss pulse and twisted in a vertically aligned homogenous magnetic field (20 Gauss) that was varying sinusoidally in time. Measurements were performed at a single frequency of 0.75 Hz. The sinusoidal twisting magnetic field caused a rotation and a pivoting displacement of the bead. As the bead moved, the cell developed internal stresses to resist the bead motion. Thus, the lateral bead displacements in response to the resulting oscillatory magnetic torque were detected optically (in spatial resolution of 5 nm), and the ratio of specific torque to bead displacements was computed and expressed as the cells stiffness in units of Pascals (Pa) per nanometer.

In order to evaluate the variation of cell stiffness in response to drug treatment, the cells were first measured for 60 s to achieve baseline stiffness. Then a drug was added to the cells, after which the cells were continuously measured for stiffness for up to 300 s. And the effect of the drug on the cell stiffness

was quantified by the ratio of the averaged plateau value of cell stiffness in the presence of the drug treatment to baseline stiffness in the absence of the drug treatment.

2.6 Assessment of Cell Traction Force of ASMCs

Cell traction force, i.e. the distribution of contractile force arising at the interface between each adherent cell and its substrate (traction field) and their change in response to increasing concentrations of contraction agonist such as His every 3 min, was measured by Fourier transform traction force microscopy (FTTM) as previously described [35]. In brief, after cells reached confluence in plastic dishes, they were serum deprived for 48 h before being trypsinized. The cells (5000 cells/dish) were then plated very sparsely in serum free medium on type I collagen (0.05 mg/ml) coated polyacrylamide gel dishes for 6 h before experiment. Subsequently, the control images of the cells and 0.2- μ m diameter fluorescent microbeads (Molecular Probes, Eugene, OR) embedded near the gel apical surface were recorded (before and after a 5-min treatment with agonist) every 40 s. After the treatments were completed, the cells were trypsinized and the cell-free bead positions were recorded as a reference point (traction-free) for bead displacement. The displacement field between a pair of images was obtained by identifying the coordinates of the peak of the cross-correlation function.

From the displacement field and known elastic properties of the gel (Young's modulus of the gel was determined to be 4,000 Pa, and a Poisson's ratio was taken to be 0.5), the traction field was calculated and used to further determine the contractile stress (pre-stress), defined as the net tensile force transmitted by the actin CSK across a cross-sectional area of the cell per unit area.

2.7 Assessment of Intracellular Calcium Concentration ($[Ca^{2+}]_i$) in ASMCs

Intracellular calcium signals were visualized using the membrane permeable $[Ca^{2+}]_i$ -sensitive fluorescent dye Fluo-4 acetoxymethylester (Fluo-4/AM), as previously described [33]. Briefly, ASMCs were inoculated into glass bottom dish for confocal microscopy (1×10^5 cell/dish). Next, cells were loaded with 5 μ M Fluo-4/AM for 30~45 min at $\sim 37^\circ\text{C}$ in a 5% CO_2 incubator. The cells were washed with Tyrode solution and incubated for 15~30 min to allow complete de-esterification of cytosolic dye. The fluorescence intensity of the cells labeled with Fluo-4/AM was measured by laser scanning confocal microscope (Zeiss LSM710; Carl Zeiss, Jena, Germany). The excitation wavelengths were set at 488 nm, and the emission wavelength was set at > 505 nm.

2.8 Statistical Analysis

Unless otherwise noted, data are presented as means \pm SEM. Results from all studies were compared using paired two ways *t*-tests. In both tests, difference with $p < 0.05$ were considered significant. An ANOVA with *t*-tests was utilized for multiple comparisons.

3 Results

3.1 Sanguinarine Altered the Morphology of ASMCs

Fig. 1(A) shows the morphology of ASMCs with or without sanguinarine. Control cells treated with vehicles had a dense sub plasma membrane layer of fibers which were long and well-organized. After treatment with sanguinarine for 12 h, the fibers inside ASMCs were disrupted in the groups treated with sanguinarine at 0.05 μ M and 0.5 μ M, so that only a few fibers could be detected. Fig. 1(B) shows the quantified cell heights, which indicates that sanguinarine also increased cell height. The average height of control cells was 2.30 ± 0.25 μ m. After treatment with sanguinarine, the cell height increased to 3.16 ± 0.45 μ m ($p < 0.05$, $n = 6$ cells per group) and 3.50 ± 0.41 μ m ($p < 0.01$, $n = 6$ cells per group) at dose of 0.05 μ M and 0.5 μ M, respectively. Fig. 1(C) shows the surface roughness of ASMC with or without sanguinarine. We observed that sanguinarine enhanced the roughness of cells both in nuclear and cell body areas.

Fig. 1(D) and 1(E) show the adhesion force and cell stiffness assessed by AFM in cultured ASMC. In control cells, we found that adhesion force and Young's modulus were larger in perinuclear areas where long fiber structure arrange systematically as compared with the nuclear area. Sanguinarine decreased the adhesion force of ASMCs at the dose of 0.005 and 0.05 μM , but increased the adhesion force at the dose of 0.5 μM . However, sanguinarine decreased the Young's modulus (stiffness) of ASMCs in all cases (both nuclear and perinuclear areas) as the dose of sanguinarine increased from 0.005-0.5 μM .

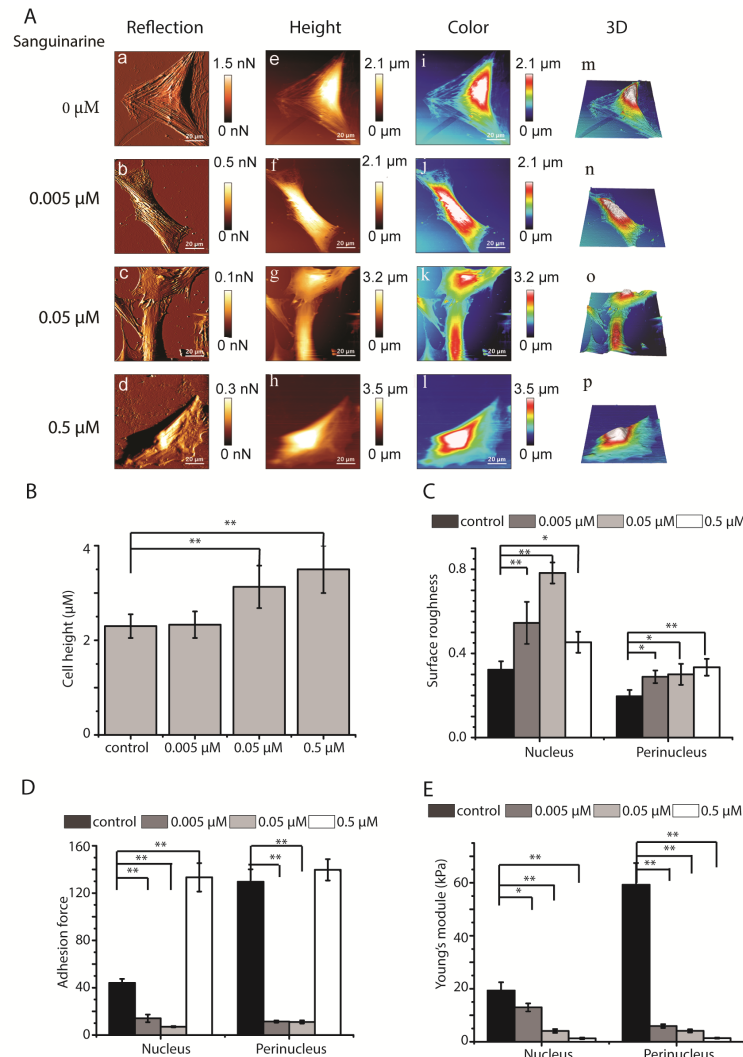


Figure 1: Sanguinarine induced alteration of morphology and mechanical properties of ASMCs as measured by AFM. (A) Representative cell surface images of living ASMCs imaged in AFM contact mode (a-d, deflection image; e-h, height image; i-l, color image; m-p, 3D image). (B) Quantification of cell height of ASMCs. (C) Surface roughness of ASMCs at nucleus and perinucleus areas. (D). Adhesion force measured by quantification of the interaction between the AFM tip and the cell membrane (E) Cell stiffness or Young's modulus calculated by fitting the force-indentation curve to Hertz model. n = 5-10 cells per group. Data are presented as means \pm SD

3.2 Sanguinarine Decreased Cell Traction Force and Cell Stiffness of ASMCs

Given the observed reduction of fibers in ASMCs due to sanguinarine, we further tested whether sanguinarine decreased contractile force and collective cell stiffness in monolayer of ASMCs. Figs. 2(a),

2(b), and 2(c) show a phase-contrast image of a rat ASMC cultured on a flexible polyacrylamide gel, the displacement of magnetic bead and corresponding traction fields computed from the bead displacement fields, respectively. Arrows in Figs. 2(b) and 2(c) show relative magnitudes and directions of the displacement and traction vector, and colors show the magnitude of the displacement and traction. In general, the greatest displacement and tractions were at the cell periphery and directed centripetally. The data in Fig. 2(d) show traction forces of AMSCs that had been treated with different doses of sanguinarine for 12 hours. The control cells (incubated with 0.1% DMSO) generated force of about 300 Pa. After treated with 0.05 and 0.5 μM of sanguinarine, ASMCs generated traction forces of 239 ± 21 Pa ($p < 0.05$ vs. control) and 226 ± 21 Pa ($p < 0.05$ vs. control) respectively.

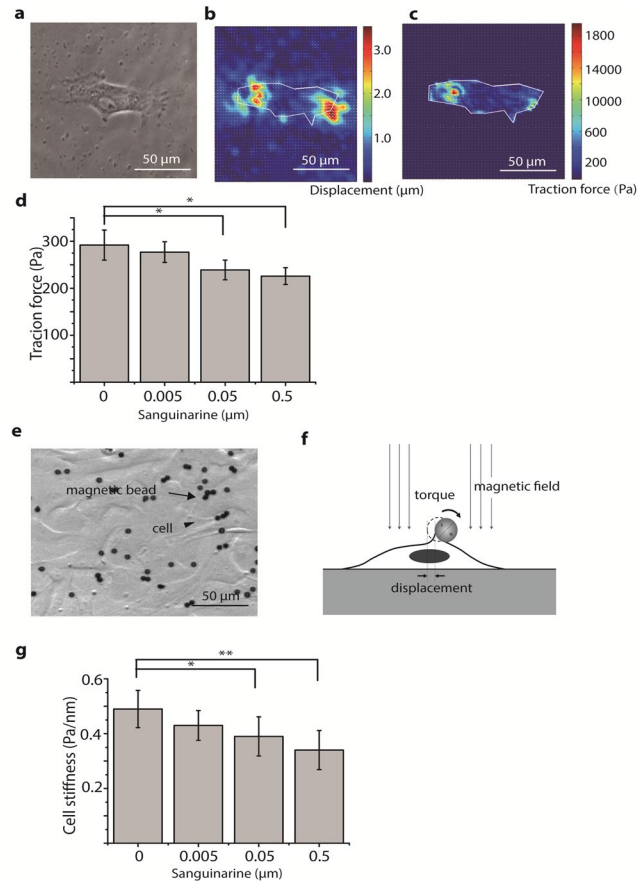


Figure 2: Sanguinarine decreased traction forces and cell stiffness of ASMCs. (a) Representative phase image of an ASMC cultured on the polyacrylamide gel coated with type I collagen. (b) The displacement field from the two fluorescent images of the microbeads in the gel (the condition with cells relative to the cell free condition). (c) Traction force of the gel. Colors show the absolute magnitude of the displacements in μm . (d) Quantified traction forces of the cells without/with sanguinarine treatment (0.005-0.5 μM , 12 h). (e) Cells with microbeads attached for OMTC. (f) Schematic illustration of OMTC principle. (g) Cell stiffness of ASMCs measured by OMTC without/with sanguinarine treatment (0.005-0.5 μM , 12 h). Data are means \pm SEM; $n = 10$ -20 cells for (d) and 50-100 cells for (g). * $p < 0.05$. All Bars = 50 μm

Fig. 2(e) shows ASMCs with magnetic beads adhered to the cells. Fig. 2(f) shows the measurement method in which a magnetic microbead was attached to the cell membrane and coupled to the cytoskeleton network inside the cell through integrin, and a magnetic field was used to apply an oscillatory mechanical torque to the bead. Cell stiffness was thus derived from the relationship between

the applied torque and the displacement of the bead due to twisting deformation of the cell. Fig. 2(g) shows the cell stiffness of ASMCs with or without sanguinarine treatment. We also found that sanguinarine decreased the stiffness of ASMCs in a dose-dependent manner.

3.3 Sanguinarine Inhibited Histamine-Increased Contraction and Stiffness of ASMCs

Fig. 3(a) shows that His increased the traction force in a dose-dependent manner. The maximum of relative traction force induced by His was about 2 fold. To test whether sanguinarine affected the response of ASMC to His, ASMCs were pretreated with sanguinarine (0.005 μ M, 0.05 μ M and 0.5 μ M) for 12 h and then exposed to 10 μ M of His. And the changes of relative traction force were shown in Fig. 3(b). The traction force of ASMCs treated with 10 μ M of His without sanguinarine increased to about 1.40 fold when compared to cells treated with vehicle (control). After pretreatment with sanguinarine (0.005 μ M, 0.05 μ M and 0.5 μ M) for 12 h, the relative traction force of ASMC exposed to 10 μ M of His decreased to 1.27 ± 0.16 , 1.12 ± 0.11 ($*p < 0.05$, vs. His group, $n = 8-10$ cells per group), and 0.86 ± 0.09 ($*p < 0.01$, vs. His group, $n = 8-10$ cells per group), respectively. These data show that sanguinarine inhibited His-increased traction force of ASMCs.

Fig. 3(c) indicate that His increased relative cell stiffness dose dependently (100 nM to 100 μ M). At the dose of 10 μ M of His, the increase of cell stiffness was about 2 fold. However, with pretreatment of sanguinarine, the relative cell stiffness of ASMC exposed to 10 μ M of His decreased to 1.59 ± 0.10 , 1.32 ± 0.08 ($*p < 0.05$, vs. His group, $n = 50-100$ cells per group), and 1.28 ± 0.04 ($*p < 0.05$, vs. His group, $n = 50-100$ cells per group). These data show that sanguinarine inhibited His-increased cell stiffness of ASMCs.

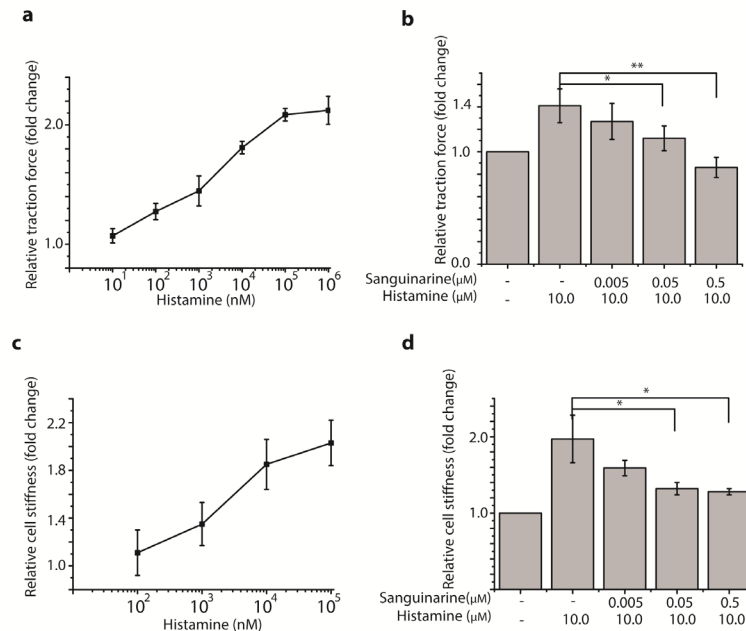


Figure 3: Sanguinarine reduced histamine-increased traction force and cell stiffness. (a) The relative traction force response of ASMCs to 3 min histamine (His) stimulation at different concentration. Relative force was defined as the fold change between baseline and post-His treatment. (b) The effect of sanguinarine on the His-increased cell traction force. (c) The relative cell stiffness response of ASMCs to 3 min His stimulation at different concentration. (d) The effect of sanguinarine on the His-increased cell stiffness. Data are means \pm SEM. Data are means \pm SEM; $n = 10-20$ cells for (a, b) and 50-100 cells for (c, d). $*p < 0.05$

3.4 Sanguinarine Inhibited Histamine-Induced Calcium Signaling in ASMCs

A key mechanism underlying His-induced contraction was to increase $[Ca^{2+}]_i$. Given sanguinarine attenuated the contractility of ASMCs induced by His, we wanted to know whether sanguinarine could attenuate the His-induced calcium signaling. To test this, the effect of sanguinarine on Ca^{2+} signaling in response to 1~1000 μM His was measured (Fig. 4(a)). The data indicated that His induced transient increase of $[Ca^{2+}]_i$ in ASMCs in a dose-dependent manner. However, exposure for 12 h to sanguinarine (0.005 μM , 0.05 μM , and 0.5 μM) significantly decreased $[Ca^{2+}]_i$ responses to His (10 μM) (Fig. 4(b)). These results show that sanguinarine decreased significantly the intracellular $[Ca^{2+}]_i$ response to His.

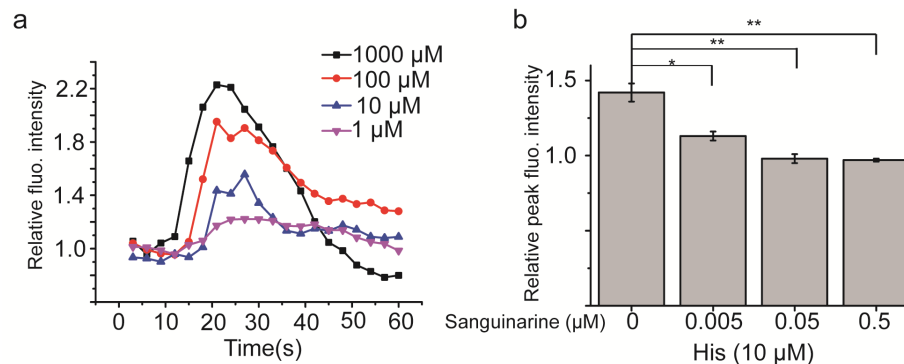


Figure 4: Sanguinarine reduced intracellular calcium signaling in ASMCs. (a) Cells were loaded with 5 μM Fluo 4-AM and intracellular calcium signaling in response to 1~1000 μM histamine (His) was measured. (b) ASMCs pre-treated for 12 h with vehicle (0.1% DMSO) or sanguinarine were loaded with 5 μM Fluo 4-AM and peak intracellular calcium signaling in response to 10 μM His was measured. Data are presented as means \pm SEM. N = 20-30 cells per group across 3 individual experiments

4 Discussion

Since mechanical properties of ASMCs are central to AHR, the changes of cellular mechanical properties comprise a logical target of therapy of asthma [25]. Existing medicines for asthma including glucocorticoids and β_2 -adrenergic agonists have a poor relationship to such a physiological endpoint. In this study, we found long term treatment of sanguinarine reduced the stiffness and traction force of ASMCs cultured in vitro. More interesting is that sanguinarine attenuated the agonist-increased cell stiffness and traction force of the ASMCs, which may lay a foundation for using sanguinarine in the prevention and treatment of asthma.

Modulation of cellular contractile forces is often the main therapeutic strategy, such as bronchodilators for airway smooth muscle cells, cardiac inotropes for cardiomyocytes, and vasodilators for vascular smooth muscle cells. For example, increased contractility to irritants in asthmatic patients shows that regulating the contractility of ASMCs is critical to control asthma [5]. As such, Park and colleague [21] invented a high-throughput platform for detecting cell contractile forces to screen asthma drugs by detecting changes in cell traction force. In this study, we found that sanguinarine attenuated the contractility response of ASMCs to His. Our findings are in line with the data from previous studies showing that sanguinarine can regulate the contractile response of smooth muscle to agonists [30]. Therefore, we extend our understanding of sanguinarine in the lung to regulate the contractility of ASMCs. The novelty of these data lies in the fact that they show a direct effect of the sanguinarine on the contractility of the ASMCs.

A major component of the signaling cascade leading to ASMC contraction is calcium [11,16]. Enhanced ASMCs intracellular Ca^{2+} response to agonist stimulation leading to increased airway constriction has been suggested to contribute to AHR. After 12 hours of treatment with sanguinarine,

peak calcium responses to stimulation with 10 μ M His were significantly attenuated. These data show that sanguinarine attenuated the excitation-contraction coupling.

The cells behave as a stress-supported tensegrity structure according to the Tensegrity Model that holds that the CSK, intracellular compression-supporting structure, is stabilized predominantly by the tensile stresses borne by fibrous structures. Wang et al. [31] have shown that the stiffness of cultured ASMCs indeed correlates linearly with the static contractile stress (also called prestress) that is born by the CSK microfilaments. Therefore, cell stiffness must increase in proportion with the level of the contractile stress. Contractile stress has an intriguing role both in maintaining structure stability of cells under mechanical loads and regulating rheological properties of CSK. In this study, we found that sanguinarine decreased the traction force (the total contractile stress) of ASMCs. These data indicate that sanguinarine can decrease the prestress of ASMCs, which in turn changes the biomechanical properties of ASMCs.

Cells not only generate prestress but also exhibit viscoelastic characteristics [8,9]. It is reported that the increased stiffness of ASMCs in asthmatic patients leads to decreased airway distensibility, which also affects the response of cells to mechanical and chemical stimuli such as stretching and agonists. Reduced airway distensibility due to increased airway stiffness and tension is a characteristic of asthma. Relaxing ASMCs increases airway distensibility in asthmatics as reflected in the improved pulmonary-conductance vs. recoil-pressure relationship. Therefore, the pathological process of asthma and airway distensibility during asthma exacerbation can be regulated by adjusting the stiffness of ASMCs [24]. In this study, we determined that sanguinarine can decrease the cell stiffness and traction force, suggesting that sanguinarine may decrease the reactivity of ASMCs to irritants.

F-actin stress fibers, key constituent of cytoskeleton, are long contractile actin bundles which affect cell morphology, structure and mechanical properties. AFM has the advantages on simultaneously obtaining the high-resolution images of the cell morphology, structures, and mechanical properties which link the relationship between morphology and mechanical properties [14]. The correlation of decreased Young's modulus values and fibers observed in topography was also observed. The results show that sanguinarine decreased the number of fibers under cell membrane and reduced Young's modulus. These above mentioned fiber structures were similar to those imaged with immunofluorescence [13,32]. Therefore, it reveals that the lower Young's moduli were associated with the decreased fibers structures, and sanguinarine may change cellular mechanical properties by disrupting stress fiber structures inside the cell.

The mechanisms may be that sanguinarine decrease protein level and/or activity of the contractility machine or reactivity of ASMCs to agonists. Sanguinarine is a specific inhibitor of protein kinase C and Rac1b [34], which can regulate cell stiffness and traction force. Additional studies have shown that sanguinarine inhibits the expression of the PKC signal of the small intestinal smooth muscle cells and inhibits the expression of M2 and M3 receptors [30]. But the specific mechanism by which sanguinarine affects the morphology and biomechanical properties of ASMCs needs to be further investigated.

Cells can be highly deformable, almost fluid-like, or maintain their structure integrity like solid [3,28]. Transition from solid-like to fluid-like cell behavior are controlled by the fibrous structures of CSK which behaves mechanically mainly as a tensed cable network. We found that the treatment of sanguinarine at high concentration decreased the stiffness and increased the adhesion force, indicating that the cell may transit from solid-like to fluid-like state in this condition.

As a well-known anti-inflammatory agent, sanguinarine suppresses IgE-mediated inflammation by inhibiting type II PtdIns 4-kinase activity and inhibits the pathway leading the activation of NF- κ signaling [7]. In this study, we also found that sanguinarine has the capability to decrease the contractility by inhibiting calcium response to His and by decreasing the cell stiffness and prestress. These data imply that sanguinarine may be a multifunctional drug for asthma therapy.

5 Conclusion

Asthma is a prevalent chronic respiratory disorder that can be debilitating for asthmatics and represents a burden for the society. Current medications control inflammation effectively but have limited

effect on regulating AHR. Using cellular mechanical properties as therapeutic target, here we showed that sanguinarine can decrease the cell stiffness and reduce the tension of ASMCs. These data together suggest that sanguinarine may have potential to be used as a drug agent for alleviation of AHR in asthma. Further study is also needed to elucidate whether sanguinarine can decrease the contractility of airway in vivo and the underlying signaling pathway mediating the pharmacological processes of sanguinarine.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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