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Effects of S100A9 in a rat model of asthma and in isolated tracheal spirals

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ABSTRACT

S100A9 is a member of the S100 family of proteins that contain two EF-hand calcium-binding motifs. We previously reported that S100A9 was differentially expressed during the early airway response phase of asthma and can be regulated by acupuncture. To understand the possible role of S100A9 in asthma, the effects of the S100A9 were investigated in a rat model of asthma and in isolated tracheal spirals. The pulmonary function and isometric tension were measured after the administration of purified recombinant S100A9. The results of *in vivo* experiments showed that S100A9 (0.1 μg/kg) significantly decreased the pulmonary resistance and increased the dynamic compliance. The *in vitro* experimental results showed that S100A9 (100, 200, 400, or 800 ng/ml, final concentrations) significantly reduced the isometric tension of isolated tracheal spirals. These results suggest that S100A9 elicits dose-dependent anti-asthmatic effects and may provide further insight into the treatment of asthma.

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1. Introduction

Asthma is a complex disease that is characterised by airway obstruction, airway hyper-responsiveness, and multi-cellular inflammation [1]. One of the hallmarks of asthma is bronchial obstruction that is caused by the constriction of the airway smooth muscle [2], which is closely associated with the alteration in calcium (Ca^{2+}) homeostasis [3] and oxidative stress [4]. Ca^{2+} ions are involved in the excitation stage of smooth muscle contraction [5], and the intracellular signalling for contraction is mediated by several Ca^{2+}-binding proteins, which include the S100 family of proteins (S100s) [6]. S100s are the largest family of EF-hand proteins, and the binding of Ca^{2+} to the S100 binding protein may elicit access of the hydrophobic binding domain of S100 to the target proteins [7]. S100s may also be involved in the scavenging of oxidants and protecting the cell or tissue from inflammation [8].

A member of the S100s, S100A9, is a small soluble protein that is highly expressed in neutrophils, activated monocytes, and early differentiated macrophages [9]. S100A9 is composed of 113 amino acids, and its molecular mass is 13 kDa. S100A9 is released into the blood or extracellular space and regulates the cellular activity of the endocrine, paracrine and autocrine systems [10] by interacting with cell surface receptors, such as the receptor for advanced glycation end products (RAGE) [11] and Toll-like receptor 4 (TLR4) [12]. The normal serum concentration of S100A9 is 338 ng/ml in humans [13] and 75 ng/ml in mice [14]. S100A9 has several immunological functions that are involved in the regulation of cellular inflammation [15], Ca^{2+}, and reactive oxygen species (ROS). S100A9 has been shown to be differentially expressed in HL60 cells treated with eicosapentaenoic acid (35 μM) at 24 h and has anti-proliferative cellular effects that are mediated through the alteration of Ca^{2+} homeostasis [16]. Extracellular S100A9 reduces ROS production [17] and has anti-inflammatory effects [8]. Previous studies by our laboratory have shown that S100A9 gene expression is significantly increased during the early airway response phase of asthma [18] and can be regulated by acupuncture [19]. S100A9 may have potential application in the treatment of asthma [20,21], but studies examining the therapeutic efficacy of S100A9 in the treatment of asthma are lacking.

To investigate the possible role of S100A9 in the treatment of asthma, recombinant S100A9 protein was generated and purified to examine its effects on the pulmonary function in the rat model of asthma and on the isometric tension of isolated tracheal spirals.

2. Materials and methods

2.1. Construction of expression vector of S100A9

The rat S100A9 gene coding sequence was amplified with a forward primer (5′-AATGGATCCATGGCTGCCAAAACAGGAT-3′) that contained a BamHI restriction site and a reverse primer (5′-AATCT CGAGCTTCCCACAGCCTTTGC-3′) that contained an XhoI restriction

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site (GenBank NM_053587). The amplified S100A9 gene product was digested with BamHI and XhoI restriction enzymes (Takara, Japan), and then the digested fragments were ligated into a pET32a bacterial expression vector (Invitrogen, USA). The constructed recombinant pET32a-S100A9 plasmid also included a thioredoxin (Trx) gene sequence that allowed for stabilisation of the small molecular weight S100A9 protein against intracellular degradation [22]. The Trx sequence was located at the N-terminal of the fusion protein so that the function of the EF-hand sequence located at the C-terminus of the S100A9 protein was not altered [23]. Insertion of the correct gene fragment was confirmed by sequencing the recombinant pET32a-S100A9 plasmid. The recombinant pET32a-S100A9 plasmid was then transfected into the *Escherichia* coli BL21 (DE3) strain (Invitrogen, USA) until the optical density at 600 nm (OD600) approached 0.6. Next, 1 mM of isopropyl-β-D-thiogalactopyranoside (IPTG) was added to induce S100A9 gene expression, and the culture was allowed to grow at 21, 25, or 37 °C for an additional 4 h to identify the most appropriate culturing temperature for protein expression. The S100A9 fusion protein was analysed with sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE).

2.2. Purification of S100A9 fusion protein

Cells were harvested (2 g wet weight of cells from 600 ml of total culture media) and re-suspended in 60 ml of NTA-10 buffer, which was composed of 20 mM Tris–HCl, 500 mM NaCl, 10% glycerol, and 10 mM imidazole at pH 7.9. The cell suspension was frozen and thawed three times and then sonicated on ice. After the cell lysate suspension was centrifuged at 12,000*g* for 15 min, the supernatant was applied to a Ni^{2+}-chelating column (7 × 25 mm) that was packed with 2 ml of Ni^{2+}-chelating resin (GE Healthcare Life Sciences, USA) and previously equilibrated with NTA-10 buffer. The baseline absorbance was determined by washing the column with NTA-10 buffer, and then the column was washed with 10, 25, 50, 100, 250, and 500 mM imidazole at a column flow rate of 2 ml/min. Elutant fractions were collected and separated via 15% SDS–PAGE. The purified S100A9 protein was desalted using an Amicon Ultra centrifugal filter (Millipore, USA) and then lyophilised.

2.3. In vivo study

2.3.1. Animals of experiments

Rats were housed in an animal facility that was approved by the Shanghai Committee for Accreditation of Laboratory Animal, and all animal experiments conformed to the regulations of the State Science and Technology Commission. Pathogen-free, male Sprague–Dawley (SD) (SLAC Laboratory Animal Co. Ltd., Shanghai, China) rats that weighed between 110 and 130 g were housed in a pathogen-free animal facility and provided with *ad libitum* food and water.

2.3.2. Rat model of asthma

Rats were randomly divided into treatment groups that each contained eight rats per group; these groups consisted of the following: normal control rats (blank); asthmatic rats (AS); asthmatic rats treated with normal saline (NS); asthmatic rats treated with 55.0 μg/kg terbutaline (TB); asthmatic rats treated with 15.0 mg/kg hydrocortisone (HC); asthmatic rats treated with 0.1, 1.0, or 10.0 μg/kg S100A9; and asthmatic rats treated with 0.1, 1.0, or 10.0 μg/kg Trx. Doses of TB and HC correspond to an equivalent clinical dose. The protocol for the SD rat model of asthma was followed as previously described [18]. Briefly, an anaesthetised rat was placed in a supine position on a wooden plate that was warmed by an incandescent lamp. A T-shaped incision was made at the upper part of the trachea, and a T-shaped cannula, which was directly attached to a heater-controlled pneumotachograph (Hans Rudolph, USA), was gently inserted into the trachea. Tidal flow was measured from the connection of the pneumotachograph to a differential pressure transducer (AutoTran, USA). The transpulmonary pressure was measured by inserting a water-filled PE-90 tube that was coupled to a pressure transducer (Jialong, Shanghai) into the oesophagus so that the tip of the water-filled PE-90 tubing was located in the mid-thorax (lower one-third of the oesophagus). Determination of the tidal flow volume was calculated by integration of the pneumotachograph tidal flow with respect to time.

2.3.3. Measurements of pulmonary functions

The selected experimental treatments were injected through an external jugular vein 10 min prior the challenge with ovum albumin (OVA). The airway responsiveness was measured from the pulmonary resistance (RL), dynamic compliance (Cdyn) and respiratory rate (RR). RL and Cdyn were calculated over a complete respiratory cycle using the integration method over airway flow, respiratory volume, and pressure, which was continuously recorded using MFLab 3.01 software (Shanghai Medical College, Fudan University). The respiratory parameters were averaged from 60-s time segments, and the maximum RL, minimum Cdyn and change in RR values were measured and calculated as the difference measured subtracted from the corresponding baseline values. After baseline values were recorded, the allergic rats were challenged with 1 ml/kg solution of 5% OVA in normal saline by injection via the external jugular vein within 10 s. The respiratory parameters were recorded for 10 min following administration of OVA.

2.4. In vitro study

2.4.1. Preparation of rat tracheal spiral

Rats that weighed 200–300 g were sacrificed. The trachea was removed and immediately placed into a Krebs'–Henseleit solution, which was composed of NaCl 118.4 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4{\cdot}7H_2O$ 1.2 mM, $NaHCO_3$ 25.0 mM, KH_2PO_4 1.2 mM, and glucose 10.1 mM. The trachea was placed into a petri dish, and the adherent connective tissue was removed. Then, the trachea was cut into a spiral. The lower end of the tracheal spiral was fixed to a support in the tissue bath that contained Krebs'–Henseleit buffer, whereas the upper end of the tracheal spiral was attached by a thread to a tension transducer (Jialong, Shanghai) with an initial load of 1.5 g. The tracheal spiral was equilibrated in Krebs'–Henseleit buffer that was gassed with 95% O_2 and 5% CO_2 for 60 min at 37 °C; the buffer was changed every 20 min.

2.4.2. The measurement of the isometric tension

The addition of 10^{-5} mol/l acetylcholine (Ach) before treatment with the pharmacological agents produced 70–75% of the maximum contraction of the tracheal spiral. A 70–75% of the maximal contractile response was chosen to avoid inducing the contractile response near the absolute maximum of the dose–response curve of the treatment of Ach. Additionally, this response allowed for an adequate extent of contraction of the tracheal spiral so that the relaxant effects of the treatments could be examined [24]. The study of the measurement of the isometric tension was divided into the following groups: normal tracheal spiral (blank); tracheal spiral pre-treated with Ach (Ach trachea); Ach trachea treated with normal saline (NS); Ach trachea treated with 800 ng/ml of terbutaline (TB, final concentration, same as below); Ach trachea treated with 800 ng/ml of hydrocortisone (HC); Ach trachea treated with 100, 200, 400, or 800 ng/ml S100A9; and Ach trachea treated with 100, 200, 400, or 800 ng/ml Trx. The tension of each treated tracheal spiral was continuously monitored with MFLab 3.01 software for 10 min, and the final value was calculated from the correspond-

ing baseline values subtracted from each recorded value. Each tracheal spiral was only used for one experiment.

2.5. Data analysis

All data are expressed as the mean ± SE. A Boxplot was used to describe the dose–response relationship of pharmacological treatment in the asthma rat model and in the isolated tracheal spiral. Statistical significance between treatment groups was calculated using One-way ANOVA followed by a Bonferroni, LSD or Games–Howell post hoc tests (depending on the data and on the hypothesis tested) [25]. P values lower than 0.05 were considered significant.

3. Results

3.1. Expression and purification of the fusion protein

The pET32a-S100A9 plasmid was transformed into *E. coli* BL21 (DE3). Expression of S100A9-Trx, which is typically low, was significantly increased in the pET32a-S100A9-transformed DE3 culture medium. The cells were grown until they reached an OD_{600} of 0.6 and then harvested. The level of protein expression at 21 °C was the most among different temperatures. The presence of a hexahistidine tag located on the carrier protein allowed for a one-step purification of the S100A9-Trx fusion protein using Ni^{2+}-chelation chromatography. SDS–PAGE was used to confirm that the S100A9-Trx fusion protein was purified. Contaminating proteins were removed by washing with 25 mM imidazole. S100A9-Trx was then eluted from the column with 100 mM imidazole. The purity of the S100A9-Trx fusion protein was at least 80% (Fig. 1).

3.2. Measurements of pulmonary functions after OVA challenge

From 2 to 7 min of the allergen-specific early airway response, sensitised rats that were treated with OVA showed an increase in the level of RL ($P < 0.05$ versus blank, Fig. 2A), the Cdyn was decreased at 4 min ($P < 0.05$ versus blank, additional file 1) and the RR was decreased at 5 min ($P < 0.05$ versus blank, additional file 2); these results indicate an positive allergen-specific early airway response to OVA.

There was no significant difference in RL between AS and NS during the 10-min study period ($P > 0.05$). There was no difference in the RL of AS treated with any of the three doses of Trx ($P > 0.05$, Fig. 2C). TB and HC significantly decreased the RL during the 3–6 min time period of the early airway response ($P < 0.05$ versus AS). The time–response curves of each of the doses of S100A9 indicate that treatment with S100A9 has a tendency to induce relaxation. A 0.1-μg/kg dose of S100A9 significantly decreased the RL during the 3–6 min time period of the early airway response ($P < 0.05$ versus AS, Fig. 2B), the 10 μg/kg dose of S100A9 significantly decreased RL at the 6 min time point of the early airway response ($P < 0.05$ versus AS), and the 1.0 μg/kg dose of S100A9 had no effect on AS ($P > 0.05$). A dose of 0.1 μg/kg S100A9 significantly increased the level of Cdyn during the 5–7 min time period of the early airway response ($P < 0.05$ versus AS).

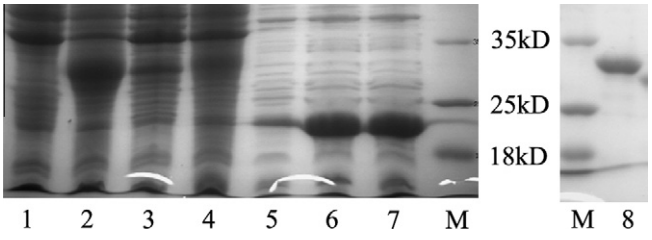

Fig. 1. SDS–PAGE analysis of fusion protein expression in BL21 (DE3) and the purified S100A9 protein. Lane 1, uninduced pET32a-S100A9 control; lane 2, induced pET32a-S100A9 at 21 °C; lane 3, induced pET32a-S100A9 at 25 °C; lane 4, induced pET32a-S100A9 at 37 °C; lane 5, pET32a empty vector; lane 6, induced pET32a empty vector at 21 °C; lane 7, induced pET32a empty vector at 25 °C; M, molecular weight marker; lane 8, purified S100A9.

Boxplot analysis of the data showed a dose–response relationship of the peak value sum of the RL during the 2–4 min time period of the early airway response (sRL_{2-4}) from each of the treatment groups (Fig. 2D). There was no significant difference in the sRL_{2-4} between the AS and NS groups ($P > 0.05$), and there was no difference in the sRL_{2-4} with the three different doses of Trx in the AS group ($P > 0.05$). Treatment with TB and HC significantly decreased the sRL_{2-4} in AS ($P < 0.05$). The sRL_{2-4} was significantly decreased in animals treated with the 0.1 μg/kg dose of S100A9 ($P < 0.05$ versus AS), and there was a significant reduction in the sRL_{2-4} when comparing to animals treated with the same dose of Trx ($P < 0.05$). A 1.0 μg/kg dose of S100A9 significantly decreased the sRL_{2-4} ($P < 0.05$ versus AS), whereas the 10.0 μg/kg dose of S100A9 showed no difference in the sRL_{2-4} when compared to the AS ($P > 0.05$ versus AS). The effects of the different doses of S100A9 were significantly different from each other ($F = 8.538$, $P < 0.01$, Fig. 2D); however, the 0.1 μg/kg dose of S100A9 elicited the greatest effect, suggesting that S100A9 causes a dose-dependent decrease in the RL in the rat model of asthma.

The relaxation rate following treatment was expressed as the percentage of difference between the sRL_{2-4} of AS. The typical relaxation that was produced by a 0.1-μg/kg dose of S100A9 was equivalent to a 67.7% suppression of the maximum contraction of AS. By comparison, a 0.1-μg/kg dose of S100A9 elicited smooth muscle relaxation that was 87.0% and 111.8% of the relaxation of measured following a 55 μg/kg dose of TB and 15.0 mg/kg dose of HC, respectively.

3.3. Measurements of isometric tension after Ach pre-contraction

Treatment of the isolated tracheal spiral with Ach significantly increased the isometric tension at the 2–5 min time period and the 9 and 10 min time period ($P < 0.05$ versus blank, Fig. 3A). There was no difference in Ach-treated tracheal spirals compared to NS ($P > 0.05$). There was no difference in the tension between the Ach- and Trx-treated tracheal spirals ($P > 0.05$, Fig. 3C). Treatment with TB and HC significantly decreased the isometric tension at the 1–5 min time period and the 1–10 min time period, respectively ($P < 0.05$ versus Ach). The time–response curve showed that treatment of the tracheal spiral with S100A9 reduced the tension of tracheal spiral over 10 min. The tension of the tracheal spirals from AS had a significant difference compared to the following treatments: tracheal spirals that were treated with 800 ng/ml S100A9 over the 10 min time period ($P < 0.05$); tracheal spirals that were treated with 100 or 200 ng/ml of S100A9 during the 2–10 min time period; and tracheal spirals that were treated with 400 ng/ml of S100A9 during the 2–4 time period and the 10 min time period ($P < 0.05$, Fig. 3B).

The dose–response relationship that describes the total tension of the treatment groups is shown in Fig. 3D. The tensions of the Ach-treated tracheal spirals were not different compared to NS tracheal spirals ($P > 0.05$) but were significantly different when compared to the blank and HC-treated tracheal spirals. All of the S100A9 doses significantly reduced the total tension ($P < 0.05$ versus Ach), whereas 200 ng/ml of S100A9 reduced the total tension of the tracheal spiral compared to the same dose of Trx ($P < 0.05$). There was a significant difference among all of the S100A9 doses ($F = 3.455$, $P < 0.05$); however, the 200 ng/ml concentration had the greatest effect on the relaxation of the tracheal spiral and

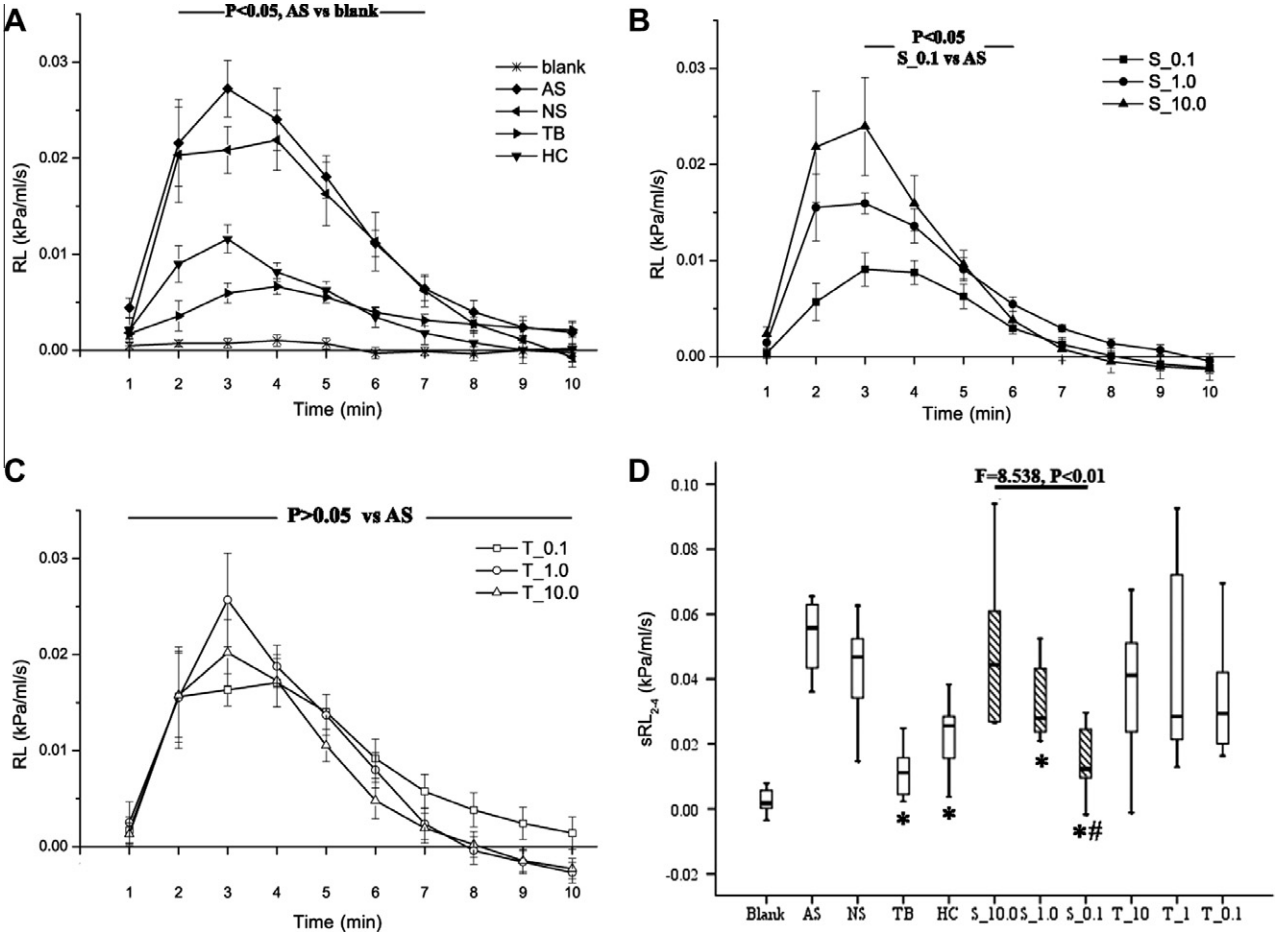

Fig. 2. Effect of S100A9 on pulmonary resistance (RL) in the rat model of asthma. (A) Time–response curves of blank, asthma (AS), terbutaline (TB), hydrocortisone (HC), and normal saline (NS) in the rat model of asthma. The RL of the AS significantly increased from 2 to 7 min when compared to the blank ($P < 0.05$). (B) Time–response curve of S100A9 doses in the rat model of asthma. A 0.1-μg/kg dose of S100A9 (S_0.1) significantly decreased the RL from 3 to 6 min when compared to the AS ($P < 0.05$). (C) Time–response curve of thioredoxin (Trx) doses in the rat model of asthma. There was no difference in the RL of AS treated with any of the three doses of Trx ($P > 0.05$). (D) Boxplot analysis of the peak value sum of the RL during the 2–4 min time period of the early airway response (sRL_{2-4}) among the treatment groups. ∗ indicates a significant difference when comparing to AS ($P < 0.05$); # indicates a significant difference when comparing to Trx at 0.1 μg/kg ($P < 0.05$). The effect of each S100A9 dose was significantly different from the other doses ($F = 8.538$, $P < 0.01$); however, the 0.1 μg/kg dose of S100A9 elicited the greatest effect.

showed a S100A9 dose-dependent decrease in the total tension in the rat tracheal spiral.

The relaxation rate of each drug treatment was expressed as the percentage of suppressions from the total contraction of the treatment on the isolated rat tracheal spiral with Ach [24]. Relaxation of the tracheal spiral that was produced by treatment with 200 ng/ml S100A9 was equivalent to a 103.5% suppression of the contraction observed in the treatment with Ach. By comparison, a 200 ng/ml dose of S100A9 produced smooth muscle relaxation that was 428.1% and 104.9% of the relaxation of measured following the 800 ng/ml dose of TB and HC, respectively.

4. Discussion

The data presented here show, for the first time, that S100A9 elicits an anti-asthmatic response in the rat model of asthma and in isolated tracheal spirals by improving pulmonary function and reducing isometric tension.

S100A9 is involved in a wide range of functions that include the regulation of Ca^{2+} homeostasis, protein phosphorylation, various enzyme activities, and protein transcription [8]. S100A9 has been shown to amplify phagocyte activation during sepsis [12] and was identified as a predictor of myocardial infarction [26]. S100A9 is significantly over-expressed in patients with inflammatory bowel disease, cystic fibrosis, rheumatoid arthritis, systemic lupus erythematosus, atherosclerosis, and allergic inflammation [27]. S100A9 is also involved in the regulation of NO and NADPH oxidase activity, which are closely related with intracellular or extracellular anti-inflammatory activity [8]. Additionally, S100A9 is involved in transcellular arachidonic acid metabolism and may regulate inflammatory responses [28]. The S100A8/A9 heterodimer inhibits *Staphylococcus aureus* growth in tissue abscesses through chelation and regulation of Mn^{2+} and Zn^{2+} [29,30].

S100s with EF-hand motifs regulate specific Ca^{2+} signalling pathways [31], and S100A9 reduces the production of ROS which stimulate smooth muscle contraction [8]. S100A9 caused a dose-dependent decrease in the RL in an asthma rat model, and treatment with a 0.1 μg/kg dose of S100A9 elicited the greatest relaxation response in isolated tracheal spirals. These results suggest that S100A9 reduces airway smooth muscle constriction by altering Ca^{2+} signalling and by reducing the production of ROS.

It has been reported that the interaction of S100A9 with several surface receptors of cell membrane induces various biological effects. In addition, S100A9 may activate the NF-κB pathway in endothelial cells through the modulation of RAGE [11]. The endogenous activation of TLR4 may be involved in the production of lethal endotoxin-induced shock [12]. Treatment of a rat tracheal spiral with S100A9 caused the dose-dependent relaxation of that

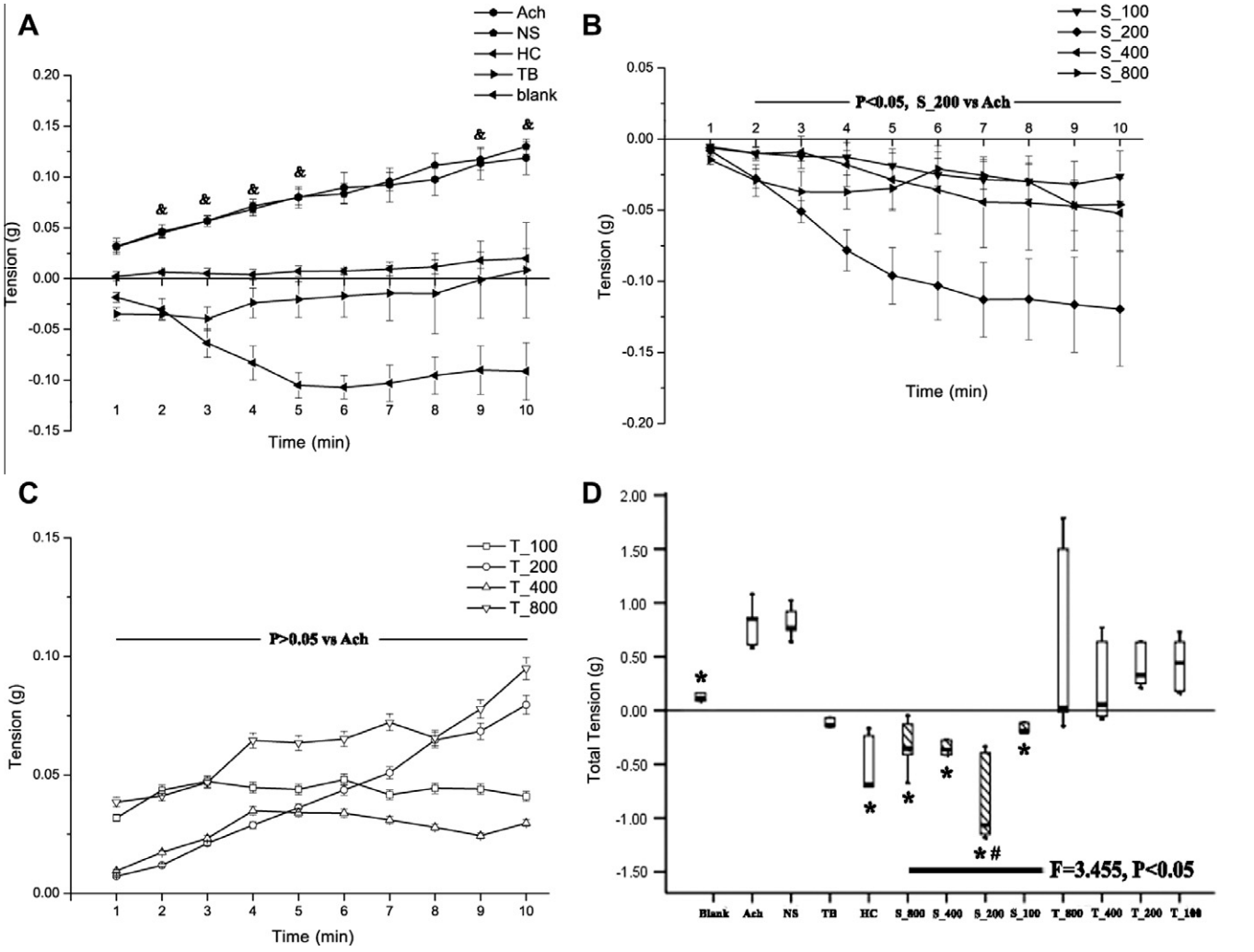

Fig. 3. Effect of S100A9 on isometric tension in isolated tracheal spirals. (A) Time–response curves of blank, acetylcholine (Ach), terbutaline (TB), hydrocortisone (HC), normal saline (NS) in isolated tracheal spiral. & indicates a significant difference between Ach and the blank at 2–5 and 9–10 min. (B) Time–response curve of S100A9 doses in isolated tracheal spirals. A 200 ng/ml dose of S100A9 (S_200) significantly decreased the tension during the 2–10 min time period when compared to Ach ($P < 0.05$). (C) Time–response curve of thioredoxin (Trx) doses in isolated tracheal spiral. There were no differences in the tension between the Ach- and Trx-treated tracheal spiral ($P > 0.05$). (D) Boxplot analysis of the sum of isometric tension among the treatment groups. * indicates a significant difference when comparing to the total contraction of Ach ($P < 0.05$), # indicates a significant difference when comparing to Trx at 200 ng/ml ($P < 0.05$). Each S100A9 dose was significantly different from the other doses ($F = 3.455$, $P < 0.05$); however, a dose of 200 ng/ml S100A9 had the greatest effect.

tracheal spiral. The addition of S100A9 to the Krebs'–Henseleit incubation solution may have enabled S100A9 to interact with surface receptors; however, further investigation is needed to understand its implications.

5. Conclusion

In the current study, we show that S100A9 improves pulmonary functions in the rat model of asthma and reduces the isometric tension in isolated tracheal spirals in a dose-dependent manner. These results highlight the potential use of S100A9 in the treatment of asthma.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.06.116.

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Table 1, the comparisons of dynamic compliance (Cdyn, ml/kPa) among groups

Cdyn	min1	min2	min3	min4	min5	min6	min7	min8	min9	min10
Blank	0.5403±1.0096	1.0922±1.626	0.4061±1.456	-0.4986±0.8638	0.18±0.7713	3.3741±1.5655	0.3927±1.145	0.9404±1.9499	1.2647±2.344	0.9735±2.2894
As	-6.991±1.947*	-15.4134±5.7202*	-23.1964±3.6001*	-24.2905±2.9705*	-23.0035±2.7822*	-19.9913±2.4906*	-16.7079±2.1635*	-12.7785±3.0593*	-11.6824±3.0045*	-7.9031±2.9849
NS	-2.6703±1.1952	-13.1198±4.6982	-22.5067±5.9616	-23.5674±4.3235	-22.5324±4.2756	-19.2606±4.1283	-15.0326±4.0648	-11.1725±3.9151	-6.9395±3.7826	-2.5115±3.9628
TB	1.9158±5.0203#	-0.0608±7.0116#	-7.0526±6.5537#	-9.0477±5.8388#	-8.792±5.2431#	-6.9443±5.0292#	-3.5474±6.7568#	-4.9042±4.1256	-4.98±3.6288	-5.2274±3.1313
HC	2.2668±2.996$	-9.9205±4.5661	-17.9959±3.2255	-17.5497±2.6598	-15.7075±2.3922	-11.2936±3.5978	-6.9029±4.5769	0.031±5.7522$	4.9935±7.2402$	6.9549±6.5464$
S_10.0	-3.348±1.7705	-17.7268±5.7917	-26.9173±4.6567	-23.4678±3.7192	-21.2402±3.0922	-16.3815±2.327	-10.946±1.9423	-7.7168±1.6292	-5.5136±1.3204	-2.6068±1.5737
S_1.0	-4.2774±1.8017	-22.9634±7.3777	-24.4368±4.0384	-24.3988±3.054	-19.998±2.5045	-15.8915±1.7239	-11.8544±1.1245	-8.0127±2.1798	-6.4748±2.3265	-2.6329±3.6179
S_0.1	-3.8251±1.23	-10.4811±3.9805	-16.8122±4.5133	-16.6172±4.2726	-12.4626±4.3422&	-7.5685±4.5183&	-5.1838±4.2723&	-2.7976±3.7452	-1.9861±3.4517	0.6381±3.8688
T_10.0	1.3045±1.8391	-9.646±3.6432	-18.5779±2.6837	-17.2684±1.7737	-13.8449±1.8527	-10.5943±2.0953	-7.1568±2.5687	-1.4038±3.2372	3.7404±4.1332	6.874±4.4412
T_1.0	-2.2136±2.8736	-4.7124±3.9374	-17.3367±4.7793	-19.481±4.9994	-18.5145±4.2728	-15.6885±3.9839	-7.6834±5.675	0.075±7.4624	3.4202±6.5867	10.3518±8.0356
T_0.1	-1.2786±1.2013	-9.4975±4.8152	-17.4278±5.9099	-16.6625±4.6623	-15.8712±4.0639	-12.496±3.8319	-9.3412±3.7227	-7.1962±3.8023	-4.8139±3.963	-2.0605±4.3832

Data were shown as mean ± SE (n=8). The values of Cdyn in the table were expressed as differential values subtracted from the corresponding baseline values. Statistical comparisons were made by ANOVA with LSD test for post hoc analysis. * indicate P<0.05, AS compared to Blank; #indicate P<0.05, AS compared to TB; $indicate P<0.05, AS compared to HC; &indicate P<0.05, S100A9 (0.1 μg/kg) compared to AS. Cdyn:dynamic compliance; AS: asthmatic rats; NS: asthmatic rats treated by Normal saline; TB: asthmatic rats treated by Terbutaline; HC: asthmatic rats treated by Hydrocortisone; S_10.0: asthmatic rats treated by S100A9 at level of 10 μg/kg; S_1.0 : asthmatic rats treated by S100A9 at level of 1.0 μg/kg; S_0.1: asthmatic rats treated by S100A9 at level of 0.1 μg/kg; T_10.0: asthmatic rats treated by Thioredoxin at level of 10 μg/kg; T_1: asthmatic rats treated by Thioredoxin at level of 1 μg/kg; T_0.1: asthmatic rats treated by Thioredoxin at level of 0.1 μg/kg.

Table 2: the comparisons of respiratory rate (RR, breaths/min) among groups

RR	min1	min2	min3	min4	min5	min6	min7	min8	min9	min10
Blank	**0.0008±1.5721**	**1.1947±1.1736**	**2.9668±1.2094**	**1.9435±1.2714**	**1.959±2.1295**	**2.8247±1.5744**	**2.7933±1.5718**	**4.1593±1.5987**	**5.7619±1.7204**	**2.6852±3.0517**
AS	**-11.774±5.9343**	**-38.4861±8.8416***	**-38.4675±5.7611***	**-38.5731±5.0669***	**-39.2018±3.6595***	**-34.9744±2.7883***	**-27.0567±3.481***	**-20.088±3.0674**	**-7.7776±10.104**	**-12.0982±5.7992**
NS	**2.3469±2.84**	**-27.8159±5.4923**	**-40.7829±7.2166**	**-34.2007±6.8215**	**-27.8896±6.4717**	**-23.5414±5.1888**	**-19.4928±4.4504**	**-16.2776±4.9415**	**-15.8549±4.5409**	**-12.4271±4.4818**
TB	**11.3588±14.8725**	**-32.998±10.3663**	**-51.7087±4.1257**	**-44.8259±3.8833**	**-37.0613±3.5541**	**-32.8557±5.0376**	**-31.8255±4.9625**	**-32.2259±5.8559**	**-29.0656±5.2551**	**-25.5375±4.385**
HC	**1.0418±5.1921**	**-33.596±9.3024**	**-35.0375±11.1233**	**-28.3659±12.5195**	**-24.177±11.9727**	**-20.1123±12.1539**	**-20.7498±11.2512**	**-21.7742±9.0014**	**-21.6535±7.8738**	**-7.2123±14.838**
S_10.0	**5.4769±7.5348**	**-30.1327±10.2272**	**-39.8108±10.9082**	**-32.0238±9.7665**	**-28.8327±8.9424**	**-22.2868±9.0249**	**-19.7792±8.3163**	**-13.7817±7.3493**	**-10.8798±7.0741**	**-9.4097±6.2863**
S_1.0	**1.8537±9.1201**	**-30.8853±12.7419**	**-32.9121±8.3414**	**-29.8563±5.8932**	**-28.0023±4.8933**	**-25.9045±4.4095**	**-21.4665±4.6041**	**-21.6857±5.9425**	**-17.4966±7.1677**	**-14.7011±7.1719**
S_0.1	**-4.0206±11.3596**	**-30.5971±6.7105**	**-33.7624±5.2254**	**-27.2607±5.0518**	**-20.3983±5.692**	**-10.9169±6.1591**	**-6.6694±6.9915**	**-3.4977±8.3218**	**-0.1095±9.202**	**0.7065±9.8516**
T_10.0	**0.3832±10.8278**	**-29.6919±14.7967**	**-43.3296±13.586**	**-42.3075±12.0694**	**-37.8292±12.8959**	**-30.3125±13.3981**	**-24.8736±13.2865**	**-18.7479±13.9047**	**-20.1434±12.5298**	**-17.4579±12.147**
T_1.0	**-8.7458±9.0513**	**-36.7625±15.2041**	**-53.2002±18.9117**	**-57.636±20.0153**	**-51.4116±17.279**	**-43.7068±17.3018**	**-32.8434±17.7603**	**-29.5762±17.2965**	**-25.7307±17.7892**	**-26.1078±17.8234**
T_0.1	**8.6616±9.472**	**-28.1604±5.2145**	**-42.0275±10.8129**	**-36.1793±6.4582**	**-30.9518±5.7392**	**-25.7716±4.7647**	**-21.2687±4.4057**	**-17.0601±3.9002**	**-11.7734±4.562**	**-11.843±4.0921**

Data were shown as mean ± SE (n=8). The values of RR in the table were expressed as differential values subtracted from the corresponding baseline values. Statistical comparisons were made by ANOVA with LSD test for post hoc analysis. * indicate $P<0.05$, AS compared to Blank. RR: respiratory rate; AS: asthmatic rats; NS: asthmatic rats treated by Normal saline; TB: asthmatic rats treated by Terbutaline; HC: asthmatic rats treated by Hydrocortisone; S_10.0: asthmatic rats treated by S100A9 at level of 10 μg/kg; S_1.0 : asthmatic rats treated by S100A9 at level of 1.0 μg/kg; S_0.1: asthmatic rats treated by S100A9 at level of 0.1 μg/kg; T_10.0: asthmatic rats treated by Thioredoxin at level of 10 μg/kg; T_1: asthmatic rats treated by Thioredoxin at level of 1 μg/kg; T_0.1: asthmatic rats treated by Thioredoxin at level of 0.1 μg/kg.