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Effects of cyclosporin A by aerosol on airway hyperresponsiveness and inflammation in guinea pigs

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KEY WORDS cyclosporins; ovalbumin; acetylcholine; histamine; eosinophils; asthma; guinea pigs

ABSTRACT

AIM: To study cyclosporin A (CsA) by aerosol for anti-asthmatic effects in guinea pigs. **METHODS**: PC_{200} changes of lung resistance (R_L) in the antigen-challenged sensitized guinea pig induced by acetylcholine (ACh) or histamine, and eosinophils changes in bronchoalveolar lavage fluid (BALF) and pulmonary histologic section induced by antigen *in vivo* in sensitized guinea pigs were investigated. **RESULTS**: Pretreatment with CsA 10 g·L^{-1} and 20 g·L^{-1} by aerosol but not with CsA 5 g·L^{-1}, dexamethasone (DXM) 0.5 mg·kg^{-1} by ip increased PC_{200} value and prevented ACh or histamine-induced airway hyperresponsiveness. However, CsA 5 g·L^{-1} also prevented histamine-induced airway hyperresponsiveness. CsA 10 g·L^{-1}, 20 g·L^{-1} and DXM 0.5 mg·kg^{-1} reduced markedly eosinophil accumulation in BALF. The lymphocyte accumulation induced by antigen was not changed significantly by CsA and DXM tested. DXM 0.5 mg·kg^{-1} increased number of neutrophil in the BALF. There was a statistical significance comparison with CsA groups. In the pulmonary histological studies, CsA 20 g·L^{-1} and DXM 0.5 mg·kg^{-1} also inhibited eosinophil infiltration in the epithelium and subepithelial connective tissue of bronchi and bronchioles. **CONCLUSION**: Anti-inflammation and anti-hyperresponsiveness of CsA by aerosol in animal model offered an experimental evidence for airway inhalation of CsA in the treatment of asthma.

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INTRODUCTION

Cyclosporin A (CsA) is a classic immunosuppressive agent for the prevention of allograft rejection and the treatment of a number of autoimmune diseases, reflecting its ability to block the transcription of cytokine genes in activated T cells. Recent studies indicate that CsA also blocks the activation of JNK and p38 signaling pathways triggered by antigen recognition, making CsA a highly specific inhibitor of T cell activation[1]. Clinical investigations revealed that oral CsA was effective in the steroid-resistance or glucocorticoid-dependent asthma treatment, but the long-term use of orally administered CsA was limited by the risk of adverse systemic effects[2,3]. These results led to investigative interest in the administration route and pharmacological mechanism of CsA. CsA suppressed interleukin-5 and GM-CSF mRNA transcription and translation by CD4$^+$ T lymphocytes in asthmatic subjects[4,5]. CsA inhibited interleukin-4 and interleukin-5 release from murine TH2-type T cells and reduced interleukin-5 mRNA abundance by inhibiting gene transcription *in vitro*[6,7]. There was a data in lung-transplant which suggested CsA by aerosol appears to be safe and effective therapy for refractory acute rejection, the efficacy achieved is considerably greater than that achieved after oral administration[8]. Inhalation of drugs in asthma improves the therapeutic index by allowing the drug to act locally within the lung but preventing systemic toxicity. Therefore, we studied inhaled CsA preparation for asthma treatment. The aim of this investigation was to evaluate whether topical inhalation of CsA into the airways could offer an alternative route of administration with efficacy in the treatment of asthma.

MATERIALS AND METHODS

Guinea pigs Hartley guinea pigs of either sex weighing 320 g ± *s* 31 g were from Laboratory Animal

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Center of Medical School of Zhejiang University (Grade Ⅱ, Certificate No 22-9601018 conferred by Zhejiang Medical Laboratorial Animal Administration Committee).

Drugs Cyclosporin A (Hangzhou Zhongmei Huadong Pharmaceutical Co Ltd), dexamethasone sodium phosphate (DXM, Suzhou Sixth Pharmaceutical Factory), urethane (Shanghai Chemical Reagent Company), histamine phosphate (Shanghai Biochemical Products Institute of Scientific Academy of China, Shanghai), egg albumin grade Ⅱ, mepyramine (Sigma, USA), acetylcholine chloride (Shanghai Third Reagent Factory), and heparin sodium (Xuzhou Biochemical Pharmaceutical Factory) were commercially available.

Sensitizing procedures Every guinea pigs was sensitized by a single im of ovalbumin 10 mg mixed with aluminium hydroxide 100 mg in saline 1 mL. These animals were used 25 – 30 d later for aerosol challenge with ovalbumin.

Treatment procedures Guinea pigs were placed in a 45 cm × 45 cm × 15 cm plastic box. Vehicle (dehydrated ethanol) and CsA solution 1 mL·min^{-1} of 5 g·L^{-1}, 10 g·L^{-1}, and 20 g·L^{-1} (In our preliminary experiments, rats were administered with CsA solution 1 mL·min^{-1} of 1.25 g·L^{-1}, 2.5 g·L^{-1}, 5 g·L^{-1}, 10 g·L^{-1}, and 20 g·L^{-1} to determine dose-effect.) was generated in an ultrasonic nebulizer (particle size 1 – 5 μm; Model 402, Heli Medical Instrumental Factory, Shanghai) for 10 min, one time every day for 6 d and 1 h before antigen challenge, and DXM as control drug 0.5 mg·kg^{-1} by intraperitoneal injection (ip).

Antigen challenge procedures On any day at d 25 – d 30, guinea pigs were placed in a 45 cm × 45 cm × 15 cm plastic box and challenged by exposure to an aerosol (1 mL·min^{-1}) of small dose antigen ovalbumin 10 mg·L^{-1} in saline which was generated in an ultrasonic nebulizer for 2 min, and repeated one time after 24 h.

Lung resistance measurement At 24 h after the second antigen challenge, guinea pigs were anesthetized with urethane (1 g·kg^{-1}, ip). The trachea was cannulated and placed in a whole body plethysmograph for the measurement of lung resistance (R_L)[9]. After 5 min for stabilizing preparation, R_L increase of airway hyperresponsiveness in the ovalbumin-challenged animals was induced with exposure to a 10-s aerosol of ACh 0.1 – 1.6 g·L^{-1} or histamine 0.01 – 0.16 g·L^{-1}. There was 5 min intervals between two concentrations. Changes in R_L to ACh or histamine administration were analyzed to construct a dose-response curve from which the ACh or histamine PC_{200} value (the concentration of ACh or histamine required to increase R_L by 200 % from the baseline). The effect of CsA was determined by comparing the ACh or histamine-induced changes in R_L after drug treatment with the mean of ACh or histamine responses alone in the same guinea pig on previous and successive control periods.

Cell counts in bronchoalveolar lavage fluids At 24 h after the second antigen challenge, guinea pigs were anesthetized with urethane (2 g·kg^{-1}, ip) and the trachea was cannulated. Bronchoalveolar lavage was performed by flushing the airways with saline 10 mL·kg^{-1} containing 1 % bovine serum albumin and 1000 kU·L^{-1} heparin sodium through the tracheal cannula. BALF was pooled and immediately centrifuged at 500 × g for 10 min at 4 ℃, the supernatant removed and the cells resuspended in 1 mL saline containing 10 % bovine serum albumin. Counts of total number of leukocytes recovered in BALF were made using a Neubauer chamber, and differential cell analysis was made under light microscope after Wright-Gieamsa staining.

Pulmonary histology The lungs of antigen-challenged guinea pig were collected intact for qualitative assessment of airway eosinophilia. The lungs were gently perfused by tracheal cannula with 10 % buffered formalin until no pleural creases were visible, and the trachea was ligated and followed by immersion in 10 % buffered formalin. The lungs were sectioned longitudinally to include trachea, airways, and both right and left lung. The tissue was paraffin embedded and sectioned at 5 μm thickness followed by hematoxylin and eosin (HE) stain. Lung eosinophilia was observed using light microscope.

Statistical analysis Data were expressed as $x \pm s$. A statistical analysis was performed using analysis of one-way analysis of variance (Student-Newman-Keuls test). PC_{200} of each animal calculated by weighed probit analysis of Bliss method.

RESULTS

Effect of cyclosporin A on airway hyperresponsiveness by acetylcholine or histamine in antigen-challenged guinea pigs There was no significant difference in basal R_L between each group (Fig 1). Inhaled acetylcholine or histamine caused a dose-related bronchoconstriction that peaked within 60 s. Pretreatment with CsA 10 g·L^{-1}, 20 g·L^{-1} by aerosol

and DXM 0.5 mg·kg^{-1} by ip, but not with CsA 5 g·L^{-1} significantly shifted acetylcholine or histamine dose-response curves to the right, increased PC_{200} value, prevented airway hyperresponsiveness (Tab 1, Fig 1). However, CsA 5 g·L^{-1} also prevented histamine-induced airway hyperresponsiveness.

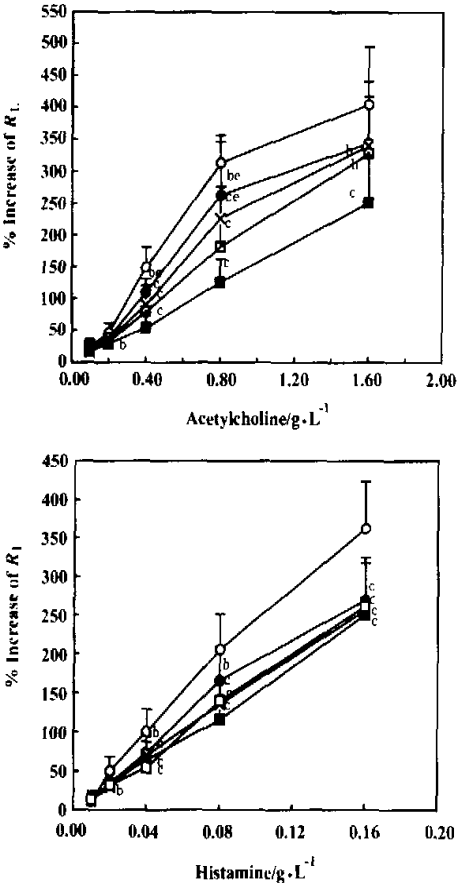

Fig 1. The inhibition of inhaled cyclosporin A on acetylcholine or histamine-induced airway hyperresponsiveness in the sensitized guinea pig. Vehicle (○), cyclosporin A 5 g·L^{-1} (●), cyclosporin A 10 g·L^{-1} (×), cyclosporin A 20 g·L^{-1} (□), dexamethasone 0.5 mg·kg^{-1}, ip (■). n = 8 guinea pigs. $x \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* vehicle. $^eP<0.05$ *vs* dexamethasone 0.5 mg·kg^{-1}.

Inhibition of antigen-induced airway inflammatory cells in BALF by cyclosporin A and dexamethasone At 24 h after the second antigen challenge, there was the accumulation of inflammatory cells in BALF. Comparison with unchallenged control animals, antigen challenge induced a pronounced increase

Tab 1. Effect of cyclosporin A and dexamethasone on airway hyperresponsiveness by acetylcholine or histamine in antigen-challenged guinea pigs. n = 8 guinea pigs. $x \pm s$. $^aP>0.05$, $^cP<0.01$ *vs* vehicle. $^eP<0.05$ *vs* dexamethasone.

Treatment	Administration route	PC_{200}/g·L^{-1} Acetylcholine	Histamine
Vehicle	aerosol	0.50 ± 0.13	0.070 ± 0.012
Cyclosporin A			
5 g·L^{-1}	aerosol	0.60 ± 0.13^{ae}	0.11 ± 0.03^a
10 g·L^{-1}	aerosol	0.86 ± 0.20^{ae}	0.12 ± 0.04^c
20 g·L^{-1}	aerosol	1.0 ± 0.3^{ae}	0.11 ± 0.04^c
Dexamethasone			
0.5 mg·kg^{-1}	ip	1.5 ± 0.6^c	0.10 ± 0.03^c

of eosinophils, lymphocytes, and neutrophils in vehicle-treated group. CsA 10 g·L^{-1}, 20 g·L^{-1}, and DXM 0.5 mg·kg^{-1} reduced markedly eosinophil accumulation in BALF (Fig 2). The lymphocyte accumulation induced by antigen was not changed significantly by any of the CsA doses and DXM tested. DXM 0.5 mg·kg^{-1} increased numbers of neutrophil in the BALF. There was a statistical significance comparison with CsA groups.

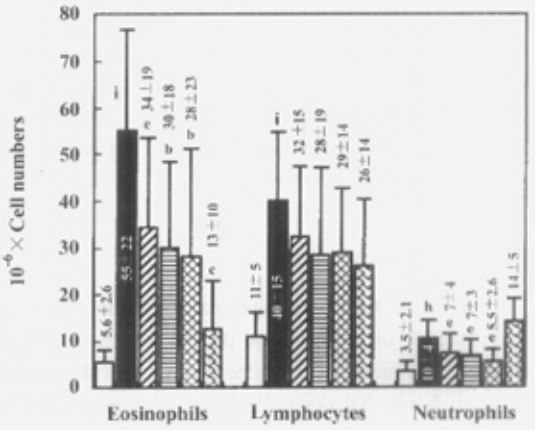

Fig 2. Inhibition of antigen-induced lung inflammatory cells in bronchoalvelar fluid by inhaled cyclosporin A. n = 8. $x \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* vehicle. $^eP<0.05$ *vs* dexamethasone 0.5 mg·kg^{-1}. $^hP<0.05$, $^iP<0.01$ *vs* unchallenged-group.

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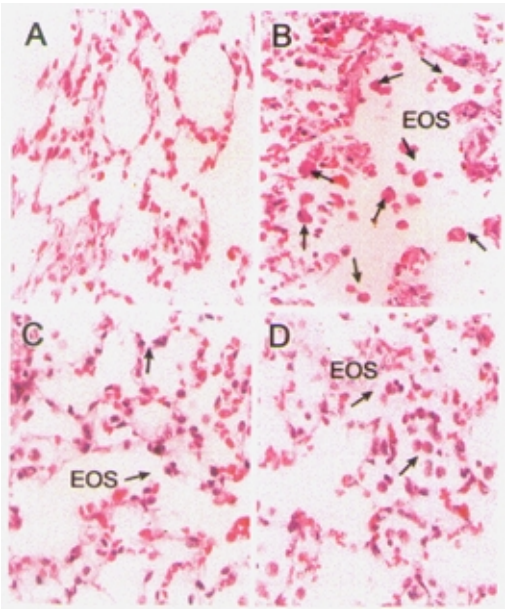

Fig 3. Reresentative sections of pulmonary histology. (A) Normal; (B) Vehicle; (C) Cyclosporin A 20 g·L^{-1}; (D) Dexamethasone 0.5 mg·kg^{-1}. HE stain, ×200. EOS: eosinophil.

Inhibition of antigen-induced histologic inflammation change in pulmonary tissue of guinea pigs by cyclosporin A and dexamethasone The eosinophil number increase of the airways of antigen-challenged guinea pigs were observated (Fig 3). Although histological quantitation of airway eosinophilia was not done in the present studies, generally increased number of eosinophils was observed in the epithelium and subepithelial connective tissue of bronchi and bronchioles. CsA 20 g·L^{-1} by aerosol and DXM (0.5 mg·kg^{-1}, ip) reduced the number of eosinophil.

DISCUSSION

Oral CsA has been reported to improve airway hyperreactivity in corticosteroid-dependent chronic severe asthma[10]. Studies of the effects of CsA in the guinea pig have shown that down-regulation of antigen-induced airways eosinophilia is not accompanied by protection against the development of airway hyperreactivity if the compound is administered orally[11]. In contrast, our study demonstrated that inhalation administration of CsA could not only decrease number of eosinophilia in BALF and lung tissue, but also prevent airway hyperreactivity in the guinea pig asthmatic model. Similar results had demonstrated that inhaled CsA inhibited airway eosinophil infiltration in the rat asthmatic model[12]. These effects of CsA on airway hypereactivity after aerosol administration are interesting and suggest that topical application might produce local effects of epithelial and smooth muscle cells which are necessary to improve lung function. Side effects in the studies in asthma were those predictable for oral CsA with an increase in diastolic blood pressure and decrease in renal function, so more and more investigators are interested in local administration system. Local administration of CsA to the lung should limit systemic distribution and reduce adverse reactions and may even enhance anti-inflammatory efficacy. Recently, a clinical study has shown that blood concentrations of CsA metered-dose inhaler (MDI) after inhalation of a single 20 mg dose was much lower than that achieved after the administration of the efficacious oral CsA dose (3 mg·kg^{-1}) for treating asthma. The highest steady-state dose (10 mg, bid) resulted in CsA concentrations that are not typically associated with systemic nephrotoxicity or immunosuppression[13]. In our studies, CsA was dissolved into dehydrated ethanol which is so rapid volatilization, that CsA become dry powder before entering airway. CsA is also potentially suitable for dry powder inhaler (DPI) according to its effective dosage. DPI can deliver a range of doses from less than 10 μg to more than 20 mg via one short inhalation. DPI represent a significant advance in pulmonary delivery technology[14].

In conclusion, our data have demonstrated that CsA by aerosol markedly inhibited airway hyperresponsiveness and inflammation in asthmatic model of guinea pig and offered an experimental evidence for topical inhalation of CsA in the treatment of asthma.

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环孢素A气雾给药对豚鼠气道高反应性和炎症的作用

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关键词 环孢菌素类；卵白蛋白；乙酰胆碱；组胺；嗜酸细胞；哮喘；豚鼠

目的：评价环孢素A气雾给药对豚鼠哮喘模型的药效. **方法**：用乙酰胆碱(ACh)或组胺诱导抗原攻击后的致敏豚鼠气道阻力 PC_{200}、支气管肺泡灌洗液(BALF)和肺组织切片中的嗜酸性粒细胞(EOS)变化观察环孢素A气雾给药后的抗气道高反应性和炎症作用. **结果**：环孢素A 10 g·L^{-1}、20 g·L^{-1}气雾给药和地塞米松 (0.5 mg·kg^{-1}, ip)增加 PC_{200}值，能预防ACh或组胺引起的气道高反应性，环孢素A 5 g·L^{-1}对组胺引起的气道高反应性也有作用，对ACh不显著. 环孢素A 10 g·L^{-1}、20 g·L^{-1}气雾给药能明显减少BALF中的EOS浸润. 与溶媒组比较，地塞米松0.5 mg·kg^{-1}增加了BALF中的中性粒细胞数目，与三组环孢素A比较有显著差异. 在肺组织学研究中，环孢素A 20 g·L^{-1}和地塞米松0.5 mg·kg^{-1}可抑制支气管和细支气管上皮和上皮表面结缔组织的EOS浸润. **结论**：环孢素A气雾吸入给药能明显对抗致敏豚鼠气道高反应性和炎症反应，为其治疗哮喘提供了一个可选择的给药途径.

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