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CORTICOSTEROID-SPARING AND BRONCHODILATING EFFECTS OF AZITHROMYCIN IN GUINEA PIGS

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ABSTRACT

The present study was designed to investigate the potential corticosteroid-sparing and bronchodilating effects of azithromycin in guinea pigs. Ovalbumin (OVA)-sensitized male guinea pigs were divided into five groups: two controls; sensitized and asthmatic; challenged with saline and OVA respectively and three asthmatic groups treated; orally in mg/kg/day; with azithromycin (6.25), dexamethasone (10) and azithromycin plus half dose dexamethasone (6.25+5) respectively for four weeks. Using double-chamber plethysmograph, PC100 methacholine was calculated as the provocative concentration of inhaled methacholine required to cause 100% increase of specific airway resistance over base line. Bronchoalveolar lavage was done for leucocytic count. Cumulative concentration responses to methacholine were conducted in isolated naïve guinea pig tracheal spiral strips after incubation with azithromycin.

All treatments significantly increased PC 100 methacholine; indicating significant decreases in airway hyperreactivity; and decreased total leucocytic and eosinophil counts compared to the control asthmatic group. The combination therapy showed more pronounced effects. Only its total leucocytic decreasing effect was more significant than that of azithromycin. Moreover, azithromycin and the combination caused significant decreases in neutrophil counts compared to the control asthmatic group while dexamethasone showed a non-significant change. In addition,

preincubation with azithromycin significantly increased the effective concentration 50 (EC_{50}) of methacholine-induced contractions of the isolated guinea pig tracheal spiral strip compared to the control naïve group.

In conclusion, azithromycin combined with half dose dexamethasone showed both antiinflammatory and bronchodilator effects in guinea pigs. Further work is needed to confirm usefulness of this combination to reduce dose of corticosteroids in asthmatic patients.

Keywords: Antiinflammatory, Asthma, Azithromycin, Dexamethasone, Methacholine

INTRODUCTION

Bronchial asthma is characterized by chronic airway inflammation, acute exacerbations and airway remodeling. The structural changes of remodeling such as subepithelial fibrosis, mucous cell hyperplasia, and increased smooth muscle mass correlate with the development of airway hyperreactivity (AHR) [1]. Patients with allergic asthma exhibit an early airway reaction (EAR) characterized by abrupt bronchoconstriction due to allergen exposure followed by a late airway reaction (LAR) that occurs 8 to 24 h later in conjunction with the airway inflammation and hyperresponsiveness. Allergen exposure causes an influx of numerous inflammatory cells and mediators into the airway [2].

Early intervention with anti-inflammatory therapy may modify the disease process. Corticosteroids are considered the most effective long-term anti-inflammatory controller medication available for asthma. They improve symptoms of asthma, however it appears that inflammation still persists at a low level [3]. The potential long-term adverse effects of corticosteroids including steroid resistance constitute a problem [4]. Macrolides; in subantimicrobial doses; have potential immunomodulatory effects beyond their antibacterial properties and thus they may become a favored add-on agent for patients who require systemic chronic therapy with corticosteroids [5]. Whether the immunomodulator effect of azithromycin and other macrolides in both respiratory and non-respiratory diseases is an individual or a class effect, it is still uncertain [6]. Compared to old members, azithromycin is a newer macrolide with better tolerability, more tissue penetration, less drug interactions, and longer half-life allowing once daily dosing [7]. The ovalbumin-sensitized guinea pig is commonly used as a small animal model of allergic asthma because it exhibits many of the characteristics observed in asthmatic patients. Guinea pigs are relatively resistant to corticosteroids compared with rats and mice,

thus high doses (3-10 mg/kg) of dexamethasone have generally been used in guinea pig models of allergic inflammation [8].

The present study was designed to investigate the hypothesis that azithromycin combined with half dose dexamethasone will inhibit the airway hyperreactivity and inflammation in ovalbumin-induced asthma in guinea pigs as effective as full dose dexamethasone. This might be useful to reduce dose and consequently adverse effects of dexamethasone in asthmatic patients.

MATERIALS AND METHODS

Animals

Male guinea pigs weighing 300-400 g were used. The study protocol was approved by the department review board and adhered to the international guidelines for the use of experimental animals. Animals were allowed free access to food and water.

Induction of Bronchial Asthma in Guinea Pigs

Sensitization of guinea pigs was done using 100 µg ovalbumin (OVA) and 100 mg aluminium hydroxide per ml saline. The mixture was gently rotated for 60 minutes to obtain a gel of which 0.5 ml was injected intraperitoneally, while the other 0.5 ml was divided equally over seven intracutaneous injection sites in the proximity of lymph nodes in the paws, lumbar regions, and neck. Sensitization with OVA induces both IgG_1 and IgE antibodies in guinea pigs; however, using aluminium hydroxide as an adjuvant causes a shift toward IgE. The sensitized animals were challenged by inhalation of 5mg/ml OVA aerosol for 15 min. once a week for four weeks. The challenge was immediately stopped if the animal showed any signs of respiratory distress. The OVA-sensitized animals were divided into five groups: two controls; sensitized and asthmatic; challenged with saline and OVA respectively and three asthmatic treated groups which received; in mg/kg/day; azithromycin (6.25), dexamethasone (10) and azithromycin plus half dose dexamethasone (6.25+5) respectively. Drugs were given by gastric gavage starting at end of the 3th week after OVA sensitization and for four weeks. Animals were exposed once weekly to OVA challenge at end of the 4th, 5th, 6th and 7th week after OVA sensitization and airway function was measured 24 h after the last challenge [9,10].

Evaluation of Airway Hyperresponsiveness (AHR) to Methacholine (MCh)

The conscious guinea pig was introduced into a whole-body double-chamber plethysmograph for measurement of the specific airway resistance (sRaw) so that the

head projects into the nasal chamber to which a nebulizer can be applied to deliver aerosol at the rate of 4.5 L/min under pressure of 1.5 bars and the body is constrained in the thoracic chamber by means of a sliding plunger. A neck cuff provides the seal between the two chambers and each chamber is equipped with a pneumotachometer in the form of six wire mesh discs. The plethysmograph is connected to a non-invasive respiratory analyzer via a pressure transducer. This transmits the respiratory flow signals (measured indirectly by changes in gas volume) to a data acquisition /analysis system (Pulmodyn Pennock software). The basis of this method is time delay between thoracic and nasal respiration (i.e. the increasing phase displacement accompanying the increasing airway resistance). This is why the volume changes in the nasal and thoracic chambers are evaluated separately. The sRaw was calculated as airway resistance multiplied by thoracic gas volume [R×V] (cm H2O/sec). R x V was displayed at 4-seconds intervals. The mean of 15 consecutive readings was calculated as the measurement at each time point [11]. Twenty four hours after the last saline or OVA challenge, guinea pigs were acclimated in the plethysmograph while breathing air for 10 min., exposed to aerosolized saline as a vehicle control for 3

min., followed by 2 min. recording interval of sRaw to determine its base-line value. Dose-response curves were generated after inhalation of doubling concentrations of MCh aerosol (6.25- 200 µg/ml) until a 100% increase in sRaw was recorded or the dose sequence was completed. PC 100 MCh was calculated as the provocative concentration of inhaled MCh required to cause 100% increase of sRaw over the base line for each animal [12].

Measurement of Leucocytic Count in Bronchoalveolar Lavage Fluid

Guinea pigs were anesthetized by intraperitoneal injection of pentobarbital (28 mg/kg). A tracheostomy was done and a cannula (23G) was introduced. The lungs were washed twice by 3 ml of sterile saline at 37° C which was recovered after 3 min. The fluid was centrifuged and the cell pellet was suspended in 1ml of saline. The total leucocytic count (TLC) was done using a haemocytometer and the differential cell count was made from films stained by Leishman's stain where 200 cells per film were counted using a microscope (Optima X5Z-H) at X400 magnification [13].

Assay of Effect of Azithromycin on MCh-Induced Contractions in the Isolated Guinea Pig Tracheal Spiral Strip

Adult male guinea pigs were used; control (naïve) and azithromycin-preincubated groups

(n=5). After killing of the animal using pentobarbital, the trachea was obtained and cut into a spiral strip of about 30 mm length and 3 mm width. The strip was mounted for isometric recording, in a 20 ml water-jacketed organ bath containing Krebs-Henseleit solution bubbled with carbogen at 37˚C. It was tied to a force displacement transducer connected to HSE bridge coupler type (570), the contraction was recorded isometrically on HSE recorder, and the resting tension was adjusted to 1g. The strip was left for 90 min. for equilibration with washouts of fresh solution every 15 min. Cumulative concentration response curves to MCh (40 nmol-10.24 µmol) were conducted after incubation of the strip with different concentrations of azithromycin (0.12, 1.2 and 12 µmol) for 20 min. **[14]**.

Statistical Analysis

It was carried out using Graphed prism, software program. Comparison between two groups was done using Student t-test. Statistical differences among more than two groups were determined using ANOVA. P values < 0.05 were considered statistically significant.

RESULTS

Effect of Treatments on Airway Hyperresponsiveness (AHR) to MCh

The control asthmatic group showed a significant decrease in PC 100 MCh (µg/ml); indicating a significant increase in AHR; compared to the control sensitized group. Pretrearment with azithromycin, dexamethasone and the combination caused significant increases in PC 100 MCh; indicating significant decreases in AHR; compared to the control asthmatic group. The effect was most; although insignificant; with the combination group **(Table 1)**.

Effect of Treatments on Leucocytic Count in Bronchoalveolar Lavage Fluid (BALF)

There were significant increases in total leucocytic, eosinophil and neutrophil counts in BALF in the control asthmatic group compared to the control sensitized group. Pretrearment with azithromycin, dexamethasone and the combination caused significant decreases of TL and eosinophil counts compared to the control asthmatic group. The combination therapy showed a more significant effect than azithromycin only regarding TLC. Regarding neutrophil count pretreatment with azithromycin and the combination caused significant decreases compared to the control asthmatic group while dexamethasone showed a non-significant change **(Table 2)**.

Effect of Azithromycin on MCh (40 nmol-10.24 µmol)-Induced Contractions in the Isolated Guinea Pig Tracheal Spiral Strip

The preincubation with different concentrations of azithromycin (0.12, 1.2 and

12 µmol) for 20 min. showed significant increases in effective concentration 50 (EC_{50}) of MCh only with the 12 µmol concentration

compared to the control naïve group (3.72 ± 0.36 *versus* 2.50 ± 0.13) **(Figure 1)**.

Table 1: Effects of Azithromycin, Dexamethasone and Azithromycin + Half Dose Dexamethasone on Airway Hyperresponsiveness; Expressed as PC100 MCh (µg/ml); in OVA-Sensitized Guinea Pigs

PC 100 MCh	Control sensitized	Control asthmatic	Azithromycin 6.25 mg/kg/day	Dexamethasone 10 mg/kg/day	Azithromycin + half dose dexamethasone (6.25 +5 mg/kg/day)
Mean ± SEM	109.50 ± 22.17	31.33 ± 7.63[*]	73.83 ± 12.85[**]	82.33 ± 4.54[**]	94.67 ± 9.19[**]

NOTE: PC100 MCh (µg/ml) is the Concentration of MCh Causing a 100% Increase of Airway Resistance Over the Baseline. *: P<0.05 Compared to Control Sensitized, **: P<0.05 Compared to Control Asthmatic

Table 2: Effects of Azithromycin, Dexamethasone and Azithromycin + Half Dose Dexamethasone on Leucocytic Count in Bronchoalveolar Lavage Fluid in OVA-Sensitized Guinea Pigs

Cell count (Mean ± SEM)	Control sensitized	Control asthmatic	Azithromycin 6.25 mg/kg/day	Dexamethasone 10 mg/kg/day	Azithromycin + half dose dexamethasone (6.25 +5 mg/kg/day)
Total leucocytic (x 10^3/cmm)	3.37 ± 0.18	13.08 ± 0.58*	6.3 ± 0.19[**]	5.45 ± 0.15[**]	4.6 ± 0.17[**,#]
Eosinophils %	4.5 ± 0.22	17.17 ± 0.87*	6.5 ± 0.22[**]	5.67 ± 0.33[**]	5.17 ± 0.31[**]
Neutrophils %	2.17 ± 0.17	4.00 ± 0.26[*]	2.67 ± 0.21[**]	3.50 ± 0.22	3.00 ± 0.26[**]

*: P<0.05 Compared to Control Sensitized, **: P<0.05 Compared to Control Asthmatic, # P<0.05 Compared to Azithromycin
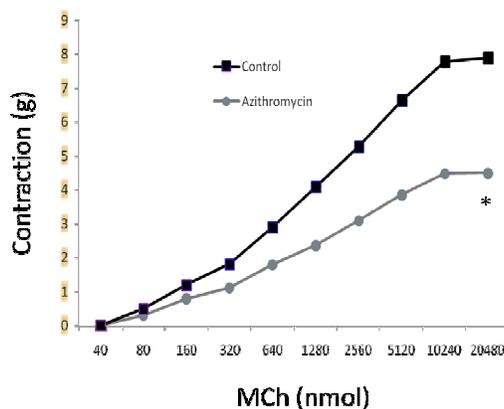

Figure 1: The Concentration-Response Curve for Methacholine (MCh, nmol) in Contractions of the Isolated Tracheal Spiral Strips (g) After Azithromycin (12 µmol) Preincubation for 20 min. (n=5)
NOTE: P<0.05 Compared to the Control Naïve Group

DISCUSSION

In the present study, all treatments significantly increased PC 100 MCh; indicating significant decreases in AHR; and

decreased total leucocytic and eosinophil counts compared to the control asthmatic group. The effects were more pronounced with the combination group. The combination

therapy showed a more significant effect than azithromycin only regarding the TLC. Moreover, pretreatment with azithromycin and the combination caused significant decreases in neutrophil count compared to the control asthmatic group while dexamethasone showed a non-significant change. In addition, preincubation with azithromycin significantly increased the EC_{50} of MCh-induced contractions of the isolated guinea pig tracheal spiral strips only with 12 μmol concentration compared to the control naïve group.

Recently, azithromycin was reported to have an anti-inflammatory effect in Th17-mediated allergic asthma in mice which may be through inhibition of inflammatory cells infiltration by reducing Th17 differentiation and decreasing secretion of inflammatory mediators [15]. In a mouse model of viral bronchiolitis, azithromycin attenuated acute and chronic airway inflammation in a way not related to its antiviral activity [16]. In asthmatic patients, long-term low-dose oral azithromycin reduced the severity of bronchial hyperresponsiveness after a histamine inhalation test [17]. In patients with bronchiolitis obliterans syndrome, azithromycin significantly reduced BAL neutrophilia and directly relaxed airways favoring combining azithromycin with

corticosteroids [18]. Patients with severe persistent asthma; treated with azithromycin open-labelly; showed clinical improvements in asthma symptoms, quality of life, and control while asthmatic patients randomized to azithromycin didn't show significant improvement in asthma outcomes, [19]. In patients with non-eosinophilic severe asthma, treatment with azithromycin for 26 weeks decreased the rate of severe exacerbations and lower respiratory tract infections requiring antibiotic treatment [20]. However, azithromycin; once daily; was ineffective in improving indices of asthma control and airway inflammation in smokers with asthma [21].

Azithromycin inhibited neutrophils infiltration and activation in patients with chronic inflammatory airway diseases, resulting in decreased mucus hypersecretion; without suppressing baseline physiologic secretions; which is a significant cause of morbidity and mortality. This may be through inhibiting the production of many proinflammatory cytokines [22]. Airway neutrophilia correlates with asthma severity due to release of many tissue-damaging mediators such as reactive oxygen species, cytokines, and neutrophil elastase which acts as a secretagogue for goblets cells causing mucus hypersecretion [23]. In OVA-

sensitized and histamine-challenged guinea pigs, dexamethasone reduced bronchial hyperresponsiveness increasing PC100 and decreased the TLC and eosinophils count markedly, but it failed to decrease the neutrophils count [24]. Corticosteroids reduce eosinophil numbers and eosinophil cationic protein levels in airways of patients with severe asthma, but they have no effect on neutrophil numbers [3]. Small dose of dexamethasone did not significantly reduce bronchial hyperresponsiveness in OVA-sensitized mice challenged with methacholine [25]. Oral administration of 500 mg azithromycin resulted in a lung concentration of 8.93 mg/L, corresponding to approximately 10 μmol of azithromycin [26]. This concentration falls within the range observed to cause the relaxant effect of azithromycin on airway smooth muscle. Thus, azithromycin incubation for 20 min. caused direct epithelium-independent relaxation of rabbit's tracheal strips precontracted with carbachol or potassium chloride. This relaxant effect was not mediated via inhibition of Ca^{2+} influx into airway smooth muscle or Ca^{2+} release from intracellular stores. It doesn't involve any alteration of Na^{+}–K^{+} ATPase activity, cAMP/cGMP formation or Rho-activated kinase pathway [14]. Attenuation of carbachol-induced contractions by

azithromycin could be important because in inflammatory diseases like asthma, mediators like tumor necrosis factor-alpha may enhance the cholinergic responses of airway smooth muscle [27].

CONCLUSION

The present study showed that azithromycin reduced airway hyperresponsiveness and inflammation in asthmatic guinea pigs due to its antiinflammatory and bronchodilator effects. These effects were more pronounced when combined with half dose dexamethasone. This might offer a chance of reducing corticosteroids' adverse effects commonly encountered with the full dose long-term use in asthmatic patients.

REFERENCES

[1] Bousquet J, Jeffery PK, Busse WW, Johnson M and Vignola A, Asthma: from bronchoconstriction to airways inflammation and remodeling, Am. J. Respir Crit. Care Med., 161, 2000, 1720-1745.

[2] Westerhof F, Timens W, Van Oosten A, Zuidhof AB, Nauta N, Schuiling M, Vos JT. W. M., Zaagsma J, Meurs H and Coers W, Inflammatory cell distribution in guinea pig airways and its relationship to airway reactivity, Mediat. Inflamm., 10, 2001, 143-154.

[3] Zeng-li W, New aspect in the treatment of asthma: targeted therapy, Chinese Med. J., 121 (7), 2008, 640-648.

[4] Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W and Thomson NC, Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma, Am. J. Respir. Crit. Care Med., 168 (11), 2003, 1308-1311.

[5] Amsden GW, Anti-inflammatory effects of macrolides—an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions?, JAC, 55 (1), 2005, 10-21.

[6] Idris SF, Chilvers ER, Haworth C, McKeon D and Condliffe AM, Azithromycin therapy for neutrophilic airways disease: myth or magic? Thorax, 64 (3), 2009, 186-189.

[7] Jaffe A, Rosenthal M, Macrolides in the respiratory tract in cystic fibrosis, J. R. Soc. Med., 95 (Suppl 41), 2002, 27-31.

[8] Nagata T, Nabe T, Fujii M, Mizutani N and Kohno S, Effects of multiple dexamethasone treatments on aggravation of allergic conjunctivitis associated with mast cell hyperplasia, Biol. Pharm. Bull., 31 (3), 2008, 464-468.

[9] Schuiling M, Meurs H, Zuidhof AB, Venema N and Zaagsma J, Dual action of iNOS-derived nitric oxide in allergen-induced airway hyperreactivity in conscious, unrestrained guinea pigs, Am. J. Respir. Crit. Care Med., 158 (5), 1998, 1442-1449.

[10] Schaafsma D, Zuidhof AB, Nelemans SA, Zaagsma J and Meurs H, Inhibition of Rho-kinase normalizes nonspecific hyperresponsiveness in passively sensitized airway smooth muscle preparations, Eur. J. Pharmacol., 531 (1-3), 2006, 145-150.

[11] Pennock BE, Cox CP, Rogers RM, Cain WA and Wells JH, A noninvasive resistance technique for measurement of changes in specific airway, J. Appl. Physiol., 46, 1979, 399-406.

[12] Duan W, Kuo IC, Selvarajan S, Chua KY, Bay BH and Fred Wong WS, Antiinflammatory effects of genistein; a tyrosine kinase inhibitor; on a guinea pig model of asthma,

Am. J. Respir. Crit. Care Med., 167, 2003, 185-192.

[13] Bonneau O, Wyss D, Ferretti S, Blaydon C, Stevenson CS and Trifilieff A, Effect of adenosine A2A receptor activation in murine models of respiratory disorders, Am. J. Physiol., 2006, 290, L1036-43.

[14] Daenas C, Hatziefthimiou A, Gourgoulianis KI and Molyvdas PA, Azithromycin has a direct relaxant effect on precontracted airway smooth muscle, Eur. J. Pharm., 553 (1-3), 2006, 280-287.

[15] Xuemei R, Yan Z, Yi H, Changzheng W, Xiaoming C and Youfan J, Azithromycin improves inflammation by suppressing functions of Th17 cells in a mouse allergic asthma model, J. Third Mil. Med. Univ., 35, 2013, 421-425

[16] Beigelman A, Mikols CL, Gunsten SP, Cannon CL, Brody SL and Walter MJ, Azithromycin attenuates airway inflammation in a mouse model of viral bronchiolitis, Respir. Res., 30 (11), 2010, 90.

[17] Ekici A, Ekici M and Frdemoglu AK, Effect of azithromycin on the severity of bronchial hyperresposiveness in patients with mild asthma, J. Asthma., 39 (2), 2002, 181-185.

[18] Verleden GM, Vanaudenaerde BM, Dupont LJ and Van Raemdonck DE**,** Azithromycin Reduces Airway Neutrophilia and Interleukin-8 in Patients with Bronchiolitis Obliterans Syndrome, Am. J. Respir Critical Care Med., 174, 2006, 566-570.

[19] Hahn DL, Grasmick M, Hetzel S and Yale S; AZMATICS (AZithroMycin-Asthma Trial In Community Settings) Study Group. Azithromycin for bronchial asthma in adults: an effectiveness trial, J. Am. Board Fam. Med., 2012, 25 (4), 2012, 442-459.

[20] Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, Verleden G, Demedts IK, Verhamme K, Delporte A, Demeyere B, Claeys G, Boelens J, Padalko E, Verschakelen J, Van Maele G, Deschepper E and Joos GF, Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial, Thorax, 68 (4), 2013, 322-329.

[21] Cameron EJ, Chaudhuri R, Mair F, McSharry C, Greenlaw N, Weir CJ,

Jolly L, Donnelly I, Gallacher K, Morrison D, Spears M, Evans TJ, Anderson K and Thomson NC, Randomised controlled trial of azithromycin in smokers with asthma, Eur. Respir. J., 42 (5), 2013, 1412-1415.

[22] Shinkai M, Henke MO and Rubin BK, Macrolide antibiotics as immunomodulatory medications: Proposed mechanisms of action, Pharmacol. Ther., 117 (3), 2008, 393-405.

[23] Carroll NG, Mutavdzic S and James AL, Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma, Thorax, 57, 2002, 677-682.

[24] Xie QM, Chen JQ, Shen WH, Yang QH and Bian RL, Effects of cyclosporine A by aerosol on airway hyperresponsiveness and

inflammation in guinea pigs, Acta. Pharmacol. Sin,. 23 (3), 2002, 243-247.

[25] Blanchet MR, Israël-Assayag E and Cormier Y, Modulation of airway inflammation and resistance in mice by a nicotinic receptor agonist, Eur. Respir. J., 26 (1), 2005, 21-27.

[26] Danesi R, Lupetti A, Barbara C, Ghelardi E, Chella A, Malizia T, Senesi S, Angeletti CA, Del Tacca M and Campa M, Comparative distribution of azithromycin in lung tissue of patients given oral daily doses of 500 and 1000 mg, J. Antimicrob. Chemother., 51, 2003, 939-945.

[27] Anticevich SZ, Hughes JM, Black JL and Armour CL, Induction of human airway hyperresponsiveness by tumour necrosis factor-alpha, Eur. J. Pharmacol., 284, 1995, 221-225.