

The anti-inflammatory effects of 1,1 dimethyl-4-phenylpiperazinium (DMPP) compared to dexamethasone in a guinea pig model of ovalbumin induced asthma

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Abstract. – BACKGROUD AND AIM: Inflammatory cells involved in the pathophysiology of asthma express nicotinic receptor. Therefore 1,1 dimethyl(-4-)phenylpiperazinium (DMPP) in two doses were compared to dexamethasone in asthmatic guinea pigs.

MATERIALS AND METHODS: Six groups were included; Normal control and five asthmatic (OVA-sensitized and challenged) groups; which were treated for 10 days as follows: two vehicles, dexamethasone (DEXA, 1 mg/kg) and DMPP (0.4 and 0.8 mg/kg) groups. Pulmonary functions and airway hyper-responsiveness were assessed. Leukocytic count, tumor necrosis factor-alpha (TNF α), interleukin-6 (IL-6) and immunoglobulin E (IgE) were measured in both blood and bronchoalveolar lavage fluid (BALF). Histopathological examination of the lung tissues was conducted.

RESULTS: Asthmatic untreated animals exhibited significant increase in early and late airway resistance (RxV) and airway hyper-responsiveness, with reduction in tidal volume. Both blood and BALF showed significant increase in total leukocytic count (TLC), eosinophils, lymphocytes, monocytes, TNF- α , IL-6 and IgE with significant decrease in neutrophils. Airway inflammatory cell infiltration and smooth muscle thickness significantly increased. DMPP 0.4 mg/kg significantly decreased late phase RXV, TLC, BALF lymphocytes, TNF- α , smooth muscle thickness and increased neutrophils in BALF over both DEXA and DMPP 0.8 mg/kg. Moreover, DMPP 0.4 mg/kg significantly decreased IL-6 and BALF eosinophils than DMPP 0.8 mg/kg and decreased serum IgE and parenchymal inflammatory infiltration than DEXA.

CONCLUNSIONS: Low dose DMPP has more anti-inflammatory effect than a high dose in most parameters and sometimes than dexamethasone. Cholinergic anti-inflammatory pathway may therefore represent a potential drug target for allergic asthma. The dose related effect of DMPP and the mechanism underlying this effect require further evaluation.

Key Words:

Asthma, Dexamethasone, DMPP, Guinea pigs, Nicotinic agonist, Guinea pigs.

Introduction

Asthma is the most common non-communicable disease. WHO estimates that 235 million people currently suffer from asthma¹. Patients with allergic asthma show, following an allergen exposure, an immediate or early-phase response characterized by abrupt onset of bronchoconstriction and a late-phase response which is associated with airway inflammatory cell influx and hyper-responsiveness^{2,3}.

The early intervention with anti-inflammatory drugs modifies the asthmatic disease process⁴. Dexamethasone is a long-acting anti-inflammatory corticosteroid controller for asthma; however due to persistence of a low level of inflammation⁵, and the well-known adverse effects of corticosteroids, other anti-inflammatory drugs should be considered⁶. The inflammatory cells involved in the pathophysiology of asthma express a variety of receptors including the nicotinic receptor (nAChR) and therefore nicotinic agonists such as 1,1-dimethyl-4-phenylpiperazinium (DMPP) can induce anti-inflammatory effects⁷. Compared to nicotine, DMPP is more hydrophilic and does not easily cross the blood-brain barrier; thus, it has minimal central effects⁸.

The ovalbumin (OVA) sensitized guinea pig is a commonly used animal model of allergic asthma because it shows many characteristics observed in asthmatic patients including early and late bronchoconstriction, nonspecific airway hyper-responsiveness, eosinophilia and plasma ex-

travasations⁹. Indeed animal models with allergen induced chronic airway inflammation are probably more relevant to study of asthma than models with a single allergen exposure¹⁰. In actively sensitized guinea pigs, antigen challenge by aerosol inhalation causes an immediate increase in specific airway resistance followed by a late response which, occurs 4-8 h after antigen challenge and continues up to 23 h later¹¹.

Acute treatment with nicotinic agonists may suppress inflammation, while chronic therapy may favor pulmonary infections due to prolonged suppression of the immune cells including macrophages¹². Consequently, this present study was designed to investigate the possible anti-inflammatory effects after treatment with a nicotine receptor agonist; DMPP for 10 days in both low and high doses, compared to; dexamethasone in a guinea pig model of asthma. The early and late phase changes in the pulmonary function and airway hyper-responsiveness to methacholine were measured. Total and differential leukocytic count, cytokines (TNF- α and IL-6) and immunoglobulin E (IgE) were assessed both in blood and bronchoalveolar lavage fluid (BALF). Moreover, lung tissues were examined histopathologically.

Materials and Methods

Drugs and Chemicals

OVA (grade III), aluminum hydroxide Al(OH)₃, methacholine (MCh), and DMPP iodide powder were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) while dexamethasone (DEXA) was a gift from ADWEA Co. (El-Obour, Egypt). OVA, AL (OH)₃, MCh and DMPP were dissolved in 0.9% (w/v) NaCl. Dexamethasone was dissolved in phosphate buffered saline (PBS).

Animals

The protocol of the study was approved by Pharmacology Ethics Committee, Faculty of Medicine, Ain Shams University, which is adhered to the international guidelines for the use of experimental animals. Thirty-six male guinea pigs (300-350 g) were used and were kept on a 12 h light/dark cycle and were allowed free access to normal chow and drinking water.

OVA Sensitization and Challenge

Sensitization of guinea pigs (on day 0 of the experiment) was done using an allergen solution containing 100 μ g OVA and 100 mg Al(OH)₃, both

were dissolved in 1.0 ml saline. The mixture was gently rotated for 60 minutes to obtain a gel of which 0.5 ml was injected intraperitoneally (i.p), while the rest was divided equally over seven intradermal injection sites in the proximity of lymph nodes in the paws, lumbar regions, and neck¹³. Sensitization with OVA induces both IgG1 and IgE antibodies in guinea pigs. However, using Al (OH)₃ as adjuvant causes a shift toward the IgE class^{14,15}.

On days 21-30 of the experiment; two hours after receiving the drug or vehicle; the sensitized animal was put into a fiberglass circular chamber (diameter = 70 cm, and height = 40 cm) connected to a nebulizer and then challenged with 0.5% (w/v) of aerosolized OVA for 10 min¹⁶. The inhalation challenge was immediately stopped if the animal showed any signs of respiratory distress, even if the animal did not complete its exposure period. Animals in the non-sensitized (normal control) group were exposed to aerosolized saline using the same protocol.

Animal Groups and Drug Administration

Six animal groups were included; 6 animal each: non-sensitized (normal control, NC; received saline injections), and five OVA-sensitized and -challenged (asthmatic) groups. The asthmatic groups were divided as follows: Two untreated groups (received saline and PBS), Dexamethasone (DEXA, 1 mg/kg)¹⁷, DMPP (0.4 & 0.8 mg/kg) groups. The dose for DMPP was selected by converting the mice dose⁸ to guinea pig dose, referring to the table of Paget and Barnes. Drugs or vehicles were given i.p. in an equal volume (1 ml) once daily for 10 days from day 21-30 of the experiment.

Pulmonary Function Measurements

Animals were placed into a double-chamber plethysmograph (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany) to accommodate for 30 min/day for three consecutive training days before the beginning of the experimental protocol¹⁸. Basal specific airway resistance was measured as described by Pennock et al¹⁹ in conscious guinea pigs 1 min. before OVA challenge, immediately (5-20 min) and late (18 h) after challenge. The plethysmograph was connected to a non-invasive respiratory analyzer via a differential pressure transducer. The specific airway resistance was calculated as airway resistance multiplied by thoracic gas volume (R $\times$ V) (cm H₂O/sec). R $\times$ V was displayed at 4-seconds intervals. The mean of 15

consecutive readings was calculated as the measurement at each time point using the Pulmodyn Pennock W software²⁰.

Effect of OVA Sensitization and Challenge on Reactivity to Methacholine (MCh)

Twenty four hours after the last saline or OVA challenge, the guinea pigs were acclimated in the plethysmograph while breathing air for 10 min, exposed to aerosolized saline for 3 minutes, followed by 2 minutes recording interval of RxV to determine its base-line value. Dose-response curves were generated after inhalation of doubling concentrations of aerosol of MCh (0.0625M-16 mM) until a 100% increase in RxV was recorded. PC 100 MCh was calculated as the concentration of MCh causing 100% increase of airway resistance over the baseline²¹.

Blood and BALF Measurements

On day 31, blood samples were collected from retro-orbital plexus under sodium pentobarbital (50 mg/Kg i.p.)²². Each sample (3.5 ml) was, then, divided into two parts. The first part (2 ml) was placed in a plain tube for separation of serum which was stored at -80 °C until measurement of cytokines (TNF α and IL-6) and IgE antibodies. The second part (1.5 ml) was placed in a heparinized tube and used for leukocyte counts. After blood collection, a tracheostomy was done a cannula was fixed under complete aseptic conditions. Sterile saline solution (10 ml) was introduced into the lungs via a 10 ml syringe and then recovered 5 min later. The recovered lavage fluid was centrifuged and the supernatant was used for determination of TNF α , IL-6, IgE and total leukocytic count. The pellet was used for differential leukocytic count¹⁸.

The total leucocytic count (TLC) was performed using an automated cell counter. For differential count, the blood sample or the recovered lavage fluid was centrifuged at 500 rpm for 10 min at 4 °C, cells in the pellet was washed in 0.5 ml saline, aliquots of the cells were spread on slides and then stained with Field's stain. After drying, 200 cells per slide were counted and cells were identified as eosinophils, neutrophils, lymphocytes and monocytes¹⁶.

Measurements of TNF α , IL-6 and IgE in serum and BALF

They were done using ELISA kits for TNF α and IL-6 (Usen Life Science, Inc., Houston, TX, USA) and IgE (AbD Serotec, Kidlington, Ox-

fordshire, UK) according to manufacturer's instructions^{23,24}.

Lung Histopathological Examination

After BALF collection, the lungs were dissected, washed with normal saline and then placed in 10% (v/v) formaldehyde solution. After fixation, lung specimens were embedded in paraffin wax, and 5- μ m sections were cut and stained with hematoxylin and eosin (Sigma-Aldrich Corp., St. Louis, MO, USA). The inflammatory cells were identified by standard morphometry and counted in five randomly chosen non-overlapping fields in tissue parenchyma. The cellular count and assessment of smooth muscle thickness of large airways were done using image analyzer (OlympusBX40, Tokyo, Japan) and Panasonic camera (GP 240 X10)¹⁶.

Statistical Analysis

The data were expressed as means $\pm$ SD and analyzed using GraphPad Prism statistical software (version 5). Because of inter-animal variability, "RxV" and "TV" values were expressed as percentage change of baseline values recorded immediately before the start of a procedure. Values of "RxV" and "TV" at baseline, 5-20 min and 18 h after OVA challenge were recorded and tabulated. Reactivity to inhaled MCh, "RxV" values logged for each MCh concentration were averaged and plotted against the MCh concentrations. The MCh provocative concentration that doubles "RxV" (MCh PC100) above the basal level were determined by linear interpolation from concentration-response curves. ANOVA was performed to compare the means of the groups and if a significant difference was obtained, Tukey's test was performed to determine differences between comparison groups. A value of $p < 0.05$ was considered to be statistically significant.

Results

There were insignificant differences among the two untreated asthmatic groups (receiving saline and PBS), thus both were pooled in one group.

Pulmonary Function

OVA-sensitized and -challenged guinea pigs exhibited early (5-10 min) and late (18h) significant increase in airway resistance (RxV) and reduction in tidal volume (TV) compared to NC

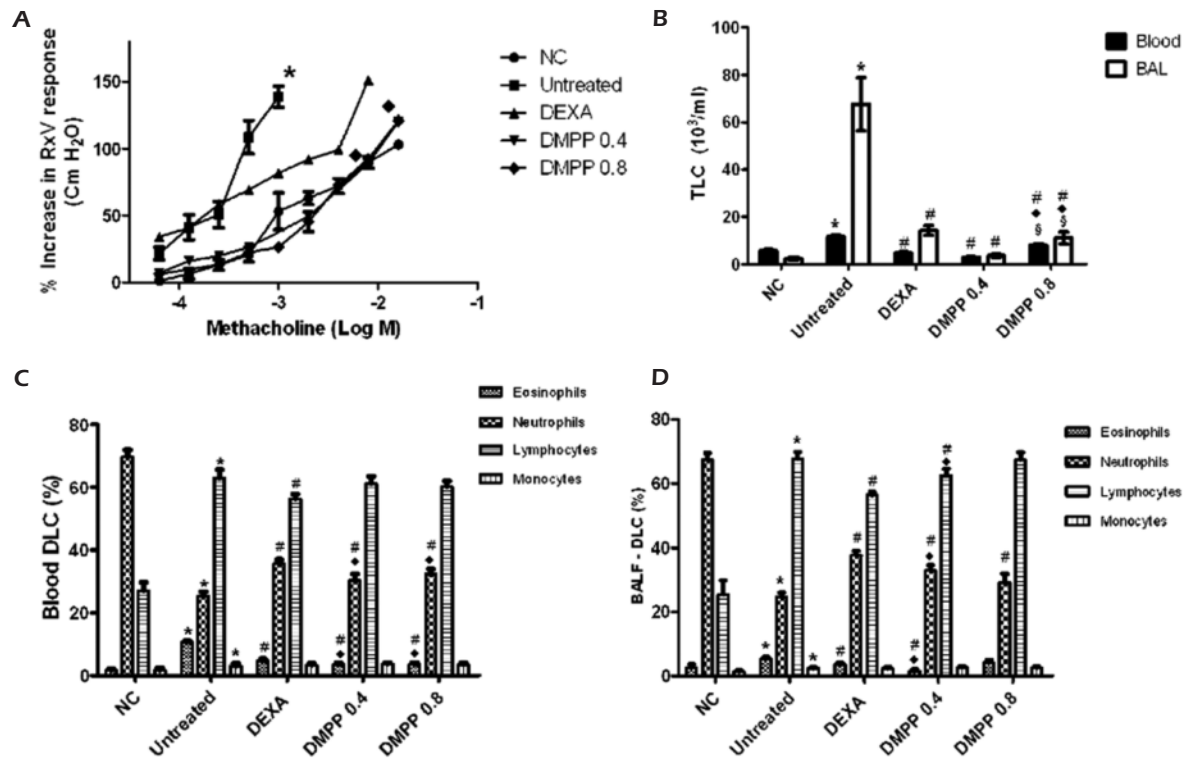


Figure 1. Effect of dexamethasone (DEXA) 1 mg/kg and 1,1-Dimethyl-4-phenyl-piperazinium (DMPP), 0.4 and 0.8 mg/kg on (A) mean % increase in airway resistance (RxV, cm H₂O), (B) total leukocytic count (TLC) in blood and BALF (bronchoalveolar lavage fluid), (C) differential leukocytic count (DLC) in blood and (D) DLC in BALF in asthmatic guinea pigs. Data are expressed as mean±SD, number of animals = 6. **p* < 0.05: compared to NC (normal control) group, #*p* < 0.05 compared to untreated group, †*p* < 0.05 compared to DEXA group and §*p* < 0.05 compared to DMPP 0.8 mg/kg group.

group. Treatments significantly improved these changes compared to untreated animals. In comparison to DEXA, the improvement was higher with both doses of DMPP in the early phase. In the late phase DMPP (0.4 mg/kg) treated animals showed a significant decrease in RxV than both DEXA-treated and DMPP (0.8 mg/kg) treated animals (Table I).

Airway Reactivity to MCh

Inhalation of MCh caused concentration-dependent increases in the RxV compared to the baseline level. In the OVA-sensitization and -challenged animals, the significant increase in airway hyper-responsiveness significantly decreased the PC100 MCh in the untreated group compared to NC group. DMPP in both doses sig-

Table I. Effect of dexamethasone (DEXA), 1 mg/kg and 1,1-Dimethyl-4-phenyl-piperazinium (DMPP), 0.4 and 0.8 mg/kg on airway resistance and tidal volume in early and late phases in asthmatic guinea pigs

Phase	Parameter	NC	Untreated	DEXA 1mg	DMPP 0.4 mg	DMPP 0.8 mg
Early	RxV (cm.H ₂ Oxsec)	1.47 ± 0.89	124.6 ± 17.1*	81.37 ± 8.68#	26.58 ± 7.14*†	32.17 ± 12.79*†
	TV(ml)	1.48 ± 0.84	-49.98 ± 11.39*	-39.00 ± 8.39#	-20.92 ± 11.42*†	-21.73 ± 7.97*†
Late	RxV (cm.H ₂ Oxsec)	1.10 ± 0.48	161.8 ± 20.81*	60.8 ± 20.30#	19.92 ± 8.86*†§	62.97 ± 22.05#
	TV(ml)	0.88 ± 0.4	-50.55 ± 9.48*	-22.07 ± 7.24#	-17.77 ± 3.26#	-24.12 ± 6.35#

RxV, airway resistance; TV, tidal volume; NC, normal control. Data are expressed as mean±SD of % change of baseline values. Number of animals = 6; **p* < 0.05: compared to NC group, #*p* < 0.05 compared to untreated group, †*p* < 0.05 compared to DEXA-treated group, §*p* < 0.05 compared to DMPP0.8mg/kg treated group.

nificantly increased the PC100 MCh over DEXA treated animals, with insignificant difference between the DMPP-treated animals (Figure 1, A).

Total and Differential Leukocytic Counts in Both Blood and BALF

As shown in Figure 1 (B, C and D) in both blood and BALF, the asthmatic animals exhibited significant increases in TLC, eosinophils, lymphocytes and monocytes with significant decrease in neutrophils compared to NC group. All treated animals exhibited a significant decrease in blood and BALF-TLC, with significant reduction at DMPP (0.4 mg/kg) group over DEXA and DMPP 0.8 mg/kg. DEXA significantly decreased blood and BALF eosinophils, lymphocytes and significantly increased neutrophils with insignificant effect on monocytes. DMPP significantly decreased blood eosinophils and increased neutrophils compared to untreated and DEXA-treated, with insignificant effect on blood lymphocytes and monocytes. In BALF, only DMPP at 0.4 mg/kg produced significant decrease of eosinophils over untreated and DMPP at 0.8 mg/kg, and significant increase of lymphocytes over the other treated groups. DMPP in both doses insignificantly affect monocytes percent with significant increase in neutrophils over untreated and DEXA treated groups, with significant effect of DMPP 0.4 mg/kg over DMPP 0.8 mg/kg.

Levels of TNF- α , IL-6 and IgE in serum and BALF

The asthmatic untreated group exhibited significant increases in levels of TNF- α , IL-6 and IgE in both serum and BALF compared to NC group while treatments significantly reversed

these changes compared to the untreated group. DMPP 0.4 mg/kg was significantly reduced TNF- α over both DEXA and DMPP 0.8 mg/kg. Meanwhile it reduced IL-6 more than DMPP 0.8 mg/kg only and reduced serum IgE more than DEXA only (Table II).

Lung Histopathology

The number of inflammatory cells (eosinophils, neutrophils, lymphocytes and monocytes) infiltrating lung parenchyma and the large airway smooth muscle thickness were significantly increased in the asthmatic groups compared to NC group. All treatments reversed the increased cell numbers more significantly in DMPP 0.4 mg/kg treated animals than DEXA-treated ones. Only DMPP 0.4 mg/kg treated animals showed a significant reduction of the smooth muscle thickness compared to untreated, DEXA- and DMPP 0.8 mg/kg treated animals (Table III and Figure 2 A-E).

Discussion

In this study the OVA-sensitized and -challenged guinea pigs exhibited a significant increase in both early and late phase airway resistance, airway hyper-responsiveness and reduction in TV compared to NC group. Moreover, in both blood and BALF, the asthmatic animals exhibited significant increases in TLC, eosinophils, lymphocytes, monocytes with significant decrease in neutrophils, in association with increase airway inflammatory cell infiltration and increase large airway smooth muscle thickness. Increased levels of TNF- α , IL-6 and IgE compared to NC group were also observed.

Table II. Effect of dexamethasone (DEXA), 1 mg/kg and 1,1-Dimethyl-4-phenyl-piperazinium (DMPP), 0.4 and 0.8 mg/kg on tumor necrosis factor alpha (TNF α), interleukin-6 (IL-6) and immunoglobulin E (IgE) levels in serum and bronchoalveolar lavage fluid (BALF) in asthmatic guinea pigs.

Parameter	NC	Untreated	DEXA 1mg	DMPP 0.4mg	DMPP 0.8mg
Serum TNF α (pg/ml)	22.10 $\pm$ 2.02	204.0 $\pm$ 10.52*	65.20 $\pm$ 8.69 [#]	52.15 $\pm$ 5.44 ^{#*}	93.88 $\pm$ 4.89 [#]
Serum IL-6 (pg/ml)	18.63 $\pm$ 2.19	327.6 $\pm$ 34.43*	83.9 $\pm$ 8.77 [#]	66.78 $\pm$ 4.85 [#]	194.1 $\pm$ 12.65 [#]
BALF TNF- α (pg/ml)	24.53 $\pm$ 3.94	358.2 $\pm$ 32.17*	112.3 $\pm$ 7.64 [#]	67.48 $\pm$ 7.65 ^{#*}	141.5 $\pm$ 11.72 [#]
BALF IL-6 (pg/ml)	64.00 $\pm$ 6.77	473.1 $\pm$ 20.65*	95.70 $\pm$ 5.86 [#]	78.23 $\pm$ 7.97 [#]	157.9 $\pm$ 9.13 [#]
Serum IgE (ng/ml)	8.13 $\pm$ 0.49	32.33 $\pm$ 3.47*	12.93 $\pm$ 1.57 [#]	9.08 $\pm$ 0.53 ^{#*}	10.82 $\pm$ 0.34 [#]
BAL IgE (ng/ml)	7.47 $\pm$ 1.36	56.93 $\pm$ 7.62*	16.90 $\pm$ 2.42 [#]	11.22 $\pm$ 0.81 [#]	15.57 $\pm$ 1.0 [#]

NC, normal control. Data are expressed as mean $\pm$ SD. Number of animals = 6; * p < 0.05: compared to NC group, [#] p < 0.05 compared to untreated group, ^{*} p < 0.05 compared to DEXA-treated group, [§] p < 0.05 compared to DMPP 0.8mg/kg treated group.

Table III. Effect of dexamethasone (DEXA) 1 mg/kg and 1,1-Dimethyl-4-phenyl-piperazinium (DMPP), 0.4 and 0.8 mg/kg on lung parenchymal inflammatory cell infiltration and large airway smooth muscle thickness in asthmatic guinea pigs.

Parameter	NC	Untreated	DEXA 1mg	DMPP 0.4mg	DMPP 0.8mg
Parenchymal inflammatory cell infiltration	63.00 ± 13.18	163.8 ± 27.49*	76.67 ± 14.15#	49.33 ± 3.88#*	71.17 ± 8.95#
Large airway smooth muscle thickness (µm)	30.63 ± 7.503	51.72 ± 10.02*	44.78 ± 11.25	35.67 ± 8.778#*	46.89 ± 14.44

NC, normal control. Data are expressed as mean ± SD. Number of animals = 6; **p* < 0.05: compared to NC group, #*p* < 0.05 compared to untreated group, **p* < 0.05 compared to DEXA-treated group, §*p* < 0.05 compared to DMPP 0.8mg/kg treated group.

DMPP administration not only produced effects which are similar to DEXA in reducing early phase changes (airway resistance and TV) and airway hyper responsiveness but also DMPP at 0.4 mg/kg produced a significant effect over DEXA in reducing late phase airway resistance.

The effect of nicotinic agonists on smooth muscles is controversial. Borjesson et al²⁵ reported that they produced smooth muscle relaxation; others reported contraction²⁶. Meanwhile Thompson et al²⁷ showed that DMPP reduced the bronchomotor tone in cats. Indeed in OVA-sensitized mice, DMPP when administered intra-nasally 10 min before MCh challenge in a dose > 3.5 mg/kg produced a decreased airway resistance that indicates a direct smooth muscle relaxant effect. An effect that could be partially explained by the observed delay and reduction in the intracellular calcium increase provoked by bradykinin⁸, given that one of the major steps of smooth muscle cell contraction is the intracellular calcium mobilization²⁸. The nAChRs are associated with phosphatidylinositol 3-kinase (PI3K) which can activate phospholipase C (PLC). Activation of PI3K and PLC depletes intracellular calcium stores^{29,30}.

In this work, DMPP as well as DEXA significantly reduced both serum and BALF IgE, but with more reduction at DMPP 0.4 mg/kg in IgE serum level. Blanchet et al⁸ demonstrated that DMPP administration in mice at 0.5 mg/kg i.p. during the sensitization period without treatment during the OVA challenges significantly reduced cell counts in BALF and serum IgE suggests that DMPP partially blocked the sensitization to OVA. This is an important point since the sensitization to allergens precedes the development of allergic asthma³¹.

IgE molecules play a crucial role in allergic respiratory diseases³². The early-phase reaction is initiated after the activation of cells bearing allergen-specific IgE and it is characterized by the

rapid activation of airway mast cells and macrophages with release of proinflammatory mediators, and reactive oxygen species (ROS), which induce contraction of airways smooth muscle, mucous secretion, and vasodilatation that end to airflow obstruction³³. Increased IgE production is considered the strongest predisposing factor for the development of asthma³⁴. Indeed, its quantity is thought to affect the intensity of the allergic reaction³⁵.

In agreement with Blanchet et al⁸ guinea pigs that received either DMPP or DEXA in the present work exhibited a decrease in the numbers of total inflammatory cells in both blood and BALF. However, as regard differential cell count, DMPP (0.4, 0.8 mg/kg) decreased blood eosinophil more than DEXA treatment but failed to decrease lymphocytes count. Furthermore, only DMPP (0.4 mg/kg) decreased BALF eosinophils and lymphocytes (over both untreated and DEXA groups). The amelioration of inflammatory cell numbers in BALF in this study was confirmed histopathologically with a decreased number of the parenchymal inflammatory cell infiltration. This may explain the improvement of airway hyper-responsiveness.

We confirmed the existence of the prominent Th₂ type cytokines TNF-α and IL-6 in the asthmatic untreated guinea pigs¹⁶, suggesting persistent airway inflammation. DMPP as well as DEXA treatment decreased the level of TNF-α, and IL-6 in BALF and in serum. This reduction in the level of cytokines correlates with the inhibition of inflammatory cell infiltration of the lung tissue. DMPP at 0.4 mg/kg produced significant decrease of serum and BALF TNF-α over DEXA. The ability of DMPP to reduce TNF-α, and IL-6 was also reported at the cellular level by Matsunaga et al³⁶; who referred that macrophage pretreatment with DMPP down regulate the production of TNF-α, and IL-6 induced by bacterial infection and this effect was antagonized com-

pletely by D-tubocurarine which indicates that DMPP effect was mediated by nAChRs. Similar data had been observed with lymphocytes and monocytes^{29,30}, which has been explained by the depletion of intracellular calcium stores following PI3K and PLC activation.

In the present study only DMPP at 0.4 mg/kg significantly reduced the large air way smooth muscle thickness, which could explain its significant reduction of the late phase airway resistance and airway hyper-responsiveness over DEXA. As increased smooth muscle thickness result in an increased resistance to airflow, particularly when there is bronchial contraction and bronchial hyperresponsiveness^{37,38}. Moreover, the ability of DMPP to reduce smooth muscle mass has been attributed to its ability to decrease the inflammatory cytokines which has a role with the repeated episodes of bronchospasm in increased smooth muscle thickness^{39,40}.

We have found that a low dose of DMPP has more effective anti-inflammatory effect than a high dose or dexamethasone. A biphasic response to DMPP was reported earlier in cell culture studies. For example, treatment of monocytes for 24 h by DMPP (0.1 to 320 μ M) significantly inhibited TNF- α production maximally at 40 μ M³⁰.

Similarly, pretreatment of eosinophils with 80, 160, or 320 μ M DMPP significantly decreased platelet-activating factor (PAF)-induced production of leukotriene C₄ (LTC₄) maximally with 160 μ M and minimally with 320 μ M and DMPP (40–320 μ M) inhibited eotaxin-induced eosinophil migration maximally with 160 μ M⁴¹.

It is worth mentioning that nicotine treatment itself produced dual effects on oxidative stress and neuroprotection, in which the effects are dependent on the differences in dosage of the drug used and their mechanisms of action. Generally, high dose of nicotine may induce neurotoxicity and stimulate oxidative stress, while reasonably low concentration may act as an antioxidant and play an important role for neuroprotective effect⁴². Barr et al⁴³ using *in vitro* mesencephalic cell model suggested that nicotine induce ROS production in a dose dependent manner and ROS in turn activate redox-sensitive transcription factor NF- κ B. ROS and NF- κ B activation may be one of the initial events transcription and expression of cytokines involved in the inflammatory response as it regulates the expression of many genes involved in immune and inflammatory response⁴⁴.

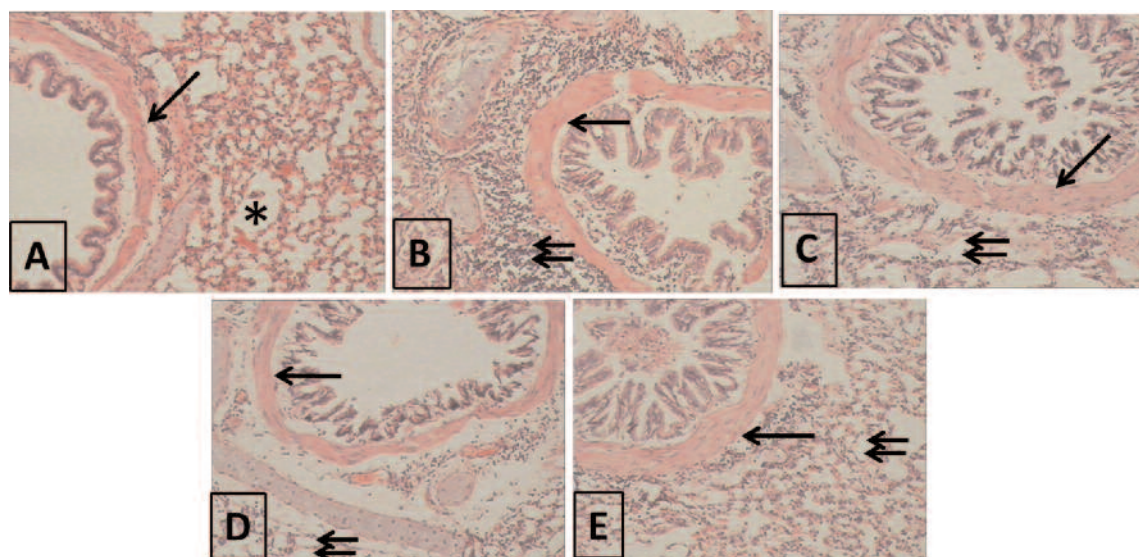


Figure 2. Photomicrographs of lung sections stained with hematoxylin and eosin using camera Panasonic GP 240 X10: **(A)**: Normal control guinea pig showing the average smooth muscle thickness (arrow) and alveolar sac (star), **(B)**: Asthmatic untreated guinea pig showing increased thickness of the smooth muscle layer (arrow) and obliteration of the alveolar sac with inflammatory cells (double arrow), **(C)**: Dexamethasone-treated guinea pig showing insignificant improvement of the smooth muscle thickness (arrow) and significant improvement of inflammatory cell infiltration (double arrow), **(D)**: DMPP (0.4 mg/kg)-treated guinea pig showing significant improvement of both the smooth muscle thickness (arrow) and the inflammatory cell infiltration (double arrow) and **(E)** DMPP (0.8 mg/kg)-treated guinea pig showing insignificant improvement on the smooth muscle thickness (arrow) and significant improvement of the inflammatory cell infiltration (double arrow).

Conclusions

DMPP (0.4, 0.8 mg/kg) mediated an anti-inflammatory effect similar to those of DEXA (1 mg/kg). Low dose DMPP (0.4 mg/kg) has more anti-inflammatory effect than a high dose. This might be valuable because it decreases immunosuppression induced by high DMPP or corticosteroids doses. The anti-inflammatory effect of DMPP is probably mediated through modulation of proinflammatory cytokines and reduction of IgE. Therefore, cholinergic anti-inflammatory pathway may represent a potential drug target for allergic asthma. However, the dose related effect of DMPP, the mechanism underlying this effect and the possible adverse effects requires further evaluation.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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