

Curcumin Attenuates Allergen-Induced Airway Hyperresponsiveness in Sensitized Guinea Pigs

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Anti-asthmatic property of curcumin (diferuloylmethane), a natural product from the rhizomes of *Curcuma longa*, has been tested in a guinea pig model of airway hyperresponsiveness. We sensitized guinea pigs with ovalbumin (OVA) to develop certain characteristic features of asthma: allergen induced airway constriction and airway hyperreactivity to histamine. Guinea pigs were treated with curcumin during sensitization (to examine its preventive effect) or after developing impaired airways features (to examine its therapeutic effect). Status of airway constriction and airway hyperreactivity were determined by measuring specific airway conductance (SGaw) using a non-invasive technique, constant-volume body plethysmography. Curcumin (20 mg/kg body weight) treatment significantly inhibits OVA-induced airway constriction ($p < 0.0399$) and airway hyperreactivity ($p < 0.0043$). The results demonstrate that curcumin is effective in improving the impaired airways features in the OVA-sensitized guinea pigs.

Key words curcumin; specific airway conductance (SGaw); airway constriction; airway hyperreactivity

Curcumin (diferuloylmethane), a natural product from the rhizomes of *Curcuma longa* is a yellow colored polyphenolic phytochemical which has been in use for a long time for the treatment of swelling, twisting and wounds. The Indian system of medicine uses curcumin, present in its raw form in the plant extract, as a wound healer and inhibitor of swelling. As a pure compound also, it has been found to possess anti-tumor, anti-cancer and anti-inflammatory activities. Several studies have clearly indicated that curcumin possesses a variety of pharmacological effects such as in wound-healing,¹⁾ anti-oxidant^{2–4)} and anti-inflammatory activities.⁵⁾ Certain recent studies indicated that curcumin may exert its anti-inflammatory properties by: a) suppressing the activation of NF-kappaB through inhibition of IKK activity,⁶⁾ b) significantly inhibiting the LPS induced proinflammatory cytokines IL-1beta and IL-8⁷⁾ and c) inhibiting iNOS production and by scavenging NO radicals.⁸⁾ There is some evidence that curcumin has anti-spasmodic effect on smooth muscle of dogs' intestine *in-vivo* and *vas-defrens* of guinea pig *in-vitro*.⁹⁾ Based on these inflammatory and anti-spasmodic properties of curcumin, we hypothesized that it could have anti-asthmatic activity as well because asthma is a chronic airway inflammatory disorder.

Asthma pathogenesis involves airway inflammation coupled with airway hyperresponsiveness (AHR) to a variety of physical and pharmacological stimuli. The incidence of asthma is on the rise globally and is reaching epidemic proportions.¹⁰⁾ The already existing remedies for asthma are known to possess detrimental side effects on prolonged use. Therefore, there is a need to explore for new anti-asthmatic agents, preferably a plant-based drug that has negligible side effects.

For our study, we selected a guinea pig model because of its marked airway reactivity and a good similarity to the airways of asthmatic human subjects.¹¹⁾ To test the effect of curcumin, we sensitized the animals with ovalbumin (OVA) to develop the characteristic features of asthma: antigen induced airway constriction and airway hyperreactivity to histamine. We report here for the first time that curcumin inhibits allergen induced airway constriction and airway hyper-

reactivity to histamine in guinea pigs.

MATERIALS AND METHODS

Animals Male guinea pigs of Dunkin-Hartley strain (National Institute of Nutrition, Hyderabad, India), 8–10 weeks old, weighing 300–350 g were used and acclimatized at least one week under laboratory conditions before conducting the experiment. Animals were allowed free access to food and water throughout the experiment. Experimental protocols were approved by the institutional ethical committee. Six groups of animals ($n=6$) were used for the study.

Sensitization Animals were sensitized with 3 intra-peritoneal (i.p.) injections of 20 μ g OVA (Sigma Chemical, Grade V, St. Louis, MO, U.S.A.) adsorbed on 10 mg alum, $Al_2(OH)_3$ in 0.5 ml 0.9% saline on alternate days. After 3 weeks of last i.p. injection, the animals were checked for airway sensitization using the technique of constant-volume body plethysmography.¹²⁾ The animals were housed in a body plethysmograph and subjected to aerosol inhalation challenge of OVA (5 mg/ml 0.9% saline through a De Vilbiss 645, nebulizer). The percent fall in the basal SGaw levels were recorded and the animals having 50% or more fall in SGaw levels were selected for further study.

Treatment with Curcumin To evaluate the preventive effect, curcumin (Central Drug House, India) (dissolved in 50% hydro-alcohol; 10, 20, 40 mg/kg body weight, 1 ml volume) or vehicle (*i.e.*, 1 ml 50% hydro-alcohol) was administered orally to each group of guinea pigs ($n=6$ in each group) daily, starting from the first day of sensitization (Fig. 1A). To examine the therapeutic effect, guinea pigs were first sensitized as described before. Guinea pigs with at least 50% fall in SGaw to OVA aerosol challenge were selected for this study. Curcumin (20 mg/kg body weight, 1 ml volume) or vehicle was administered orally to two groups ($n=6$ in each) of guinea pigs respectively, daily from day 30 to 36 (Fig. 1B). The treatment of curcumin even up to 40 mg/kg body weight to normal guinea pigs did not show any effect on SGaw values.

Measurement of Specific Airway Conductance (SGaw)

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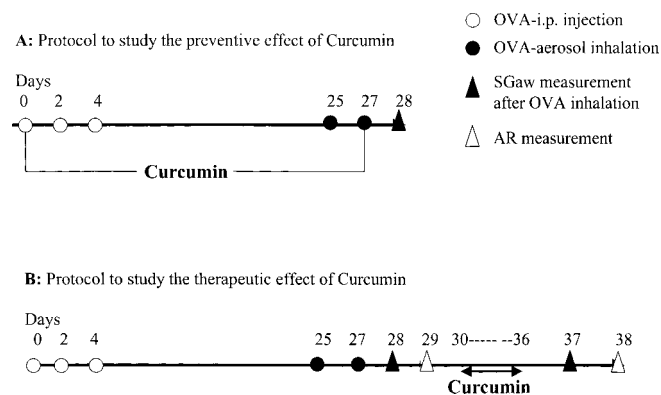


Fig. 1. (A) Protocol to Study the Preventive Effect of Curcumin

To test the preventive effect of curcumin, guinea pigs were sensitized with three intraperitoneal injections of ovalbumin (OVA; 20 µg/ml in normal saline) on days 0, 2 and 4 followed by aerosolized OVA (5 mg/ml) inhalation on days 25th and 27th. Airway conductance was measured on 28th day. The animals were orally administered with different doses of curcumin throughout this study.

(B) Protocol to Study the Therapeutic Effect of Curcumin

To test the therapeutic effect of the drug, guinea pigs were sensitized as above. After confirmation of sensitization on day 28 and AHR on day 29, curcumin was orally administered to the animals from day 30 to 36. The animals were given aerosolized OVA inhalation on day 37 and airway conductance and airway reactivity were measured on days 37 and 38 respectively.

SGaw is a measure of airway function and was measured by a non-invasive technique, constant-volume body plethysmograph as described by Agrawal.¹²⁾

Measurement of OVA-Induced Immediate and Late Airway Responses OVA-induced immediate airway response (airway constriction) was determined in the term of SGaw fall due to OVA aerosol challenge as compared to its basal SGaw value. Each individual guinea pig was fitted in the body plethysmograph and subjected to aerosol vehicle (0.9% saline) inhalation generated in a nebulizer (DeVilbiss 645, U.S.A.) at 6 l/min for 60 s via a port in the chamber and the initial SGaw was noted. The guinea pig was then given 1 ml aerosolized OVA (5 mg/ml in saline) inhalation and the final SGaw value was noted. The difference in the initial and final values gave the measure of SGaw fall and termed as airway constriction.

The late airway response due to OVA inhalation was determined as airways reactivity to histamine after 24 h of the last OVA aerosol inhalation. Histamine was dissolved in PBS and different concentrations (0.31, 0.625, 1.25, 2.5, 5.0, 10, 20 mg/ml) were prepared. Each individual guinea pig was housed in the plethysmograph and aerosol of 1 ml PBS (as vehicle) was given for 60 s to record the baseline SGaw. Similarly, aerosol of different concentrations of histamine were given in increasing order until the SGaw levels decreased approximately to 40% of the basal level. The time gap of 5 min between two doses was kept to see the effect of each delivered dose of histamine. The aerosol was generated using the same nebulizer at a 6 l/min air current. Histamine dose-SGaw curve was prepared for each individual guinea pig as described by Agrawal¹²⁾ and the dose, which produced 50% decrease in the basal SGaw level (Hist PD₅₀), was derived by linear interpolation of the curve and was used as an index of airway reactivity to histamine.

Statistics We compared the percentage SGaw values of curcumin treated groups with those of sensitized group. Using 'Kolmogorov-Smirnov Test', we found the data nor-

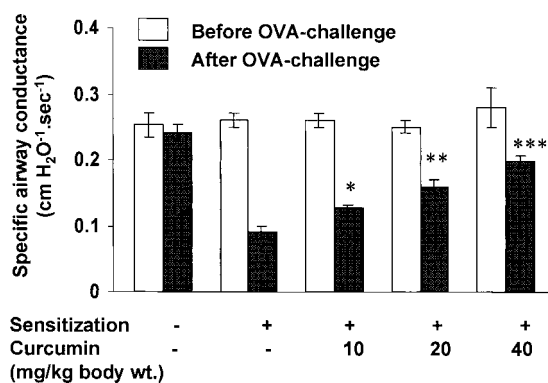


Fig. 2. Curcumin Reduces Airway Constriction in Ovalbumin-Sensitized Guinea Pigs

Experiments were performed with four groups of guinea pigs (n=6) as described in Fig. 1 legend. Briefly, guinea pigs were treated with three different doses of curcumin (10, 20, 40 mg/kg body weight) during sensitization. A vehicle treated sensitized group was kept as control. The fall in specific airway conductance in response to aerosolized OVA inhalation was measured using constant-volume body plethysmography in all the groups of guinea pigs. Results are shown as the mean ± S.E.M. Students t-test was applied to determine the significant differences of the means. *p=0.019, **p=0.0009, ***p=0.00005 indicates the difference from sensitized control.

mally distributed and hence applied Students t-test for the difference. The data are presented as mean ± S.E.M. A p value <0.05 was considered significant.

RESULTS

Curcumin Prevents OVA-Induced Airway Constriction

To determine the preventive effect, guinea pigs were treated with curcumin (10, 20, 40 mg/kg body weight) or vehicle during sensitization, as described in the Materials and Methods. Following OVA aerosol challenge, OVA-sensitized guinea pigs treated with vehicle showed a marked fall in SGaw and reached down up to 35% (p<0.00001) of its basal value, whereas sham-sensitized guinea pigs showed no change (Fig. 2). We compared the percentage SGaw values of curcumin treated groups with that of sensitized group and found that curcumin treatment significantly prevented OVA-induced fall of SGaw in a dose dependent manner (10 mg/kg, 48%, p=0.019; 20 mg/kg 64%, p=0.0009; 40 mg/kg, 70%, p=0.00005 vs. 35%, vehicle treated sensitized group). If we compare the changes in SGaw in between the drug groups, 20 mg showed a significant change over 10 mg (p=0.04); however, there was no significant effect of 40 mg/kg dose over 20 mg/kg (p=0.19). Therefore, we selected 20 mg/kg body weight for the further therapeutic study.

Curcumin Reversed OVA-Induced Airway Constriction and Airway Hyperreactivity

To determine the therapeutic effect, guinea pigs were first sensitized and then treated with curcumin, 20 mg/kg body weight for one week as described in the Materials and Methods (Fig. 1B). Curcumin was found to reverse OVA-induced fall in SGaw levels upto 68% (p=0.0399) as compared to vehicle treated sensitized group of guinea pigs (35%) (Fig. 3).

To evaluate the effect of curcumin on OVA-induced airway hyperreactivity, we determined Hist PD₅₀ values 24 h after last OVA-challenge on day 29 at sensitization and after curcumin treatment on day 38 as described in the protocol (Fig. 1B). As shown in Fig. 4, there was a significant decrease in the Hist PD₅₀ values in OVA-challenged sensitized guinea

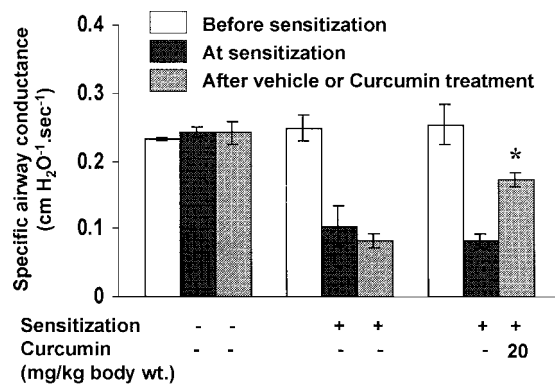


Fig. 3. Curcumin Reverses the Developed Airway Constriction

Guinea pigs were sensitized and treated with curcumin (Protocol 1B). A vehicle treated sensitized group was kept as control. The fall in specific airway conductance in response to aerosolized OVA-inhalation was measured as described in Fig. 2 legend. Results are shown as the mean \pm S.E.M. Students *t*-test was applied to determine the significance between the groups. * $p < 0.0399$ indicates the difference from vehicle treated sensitized control.

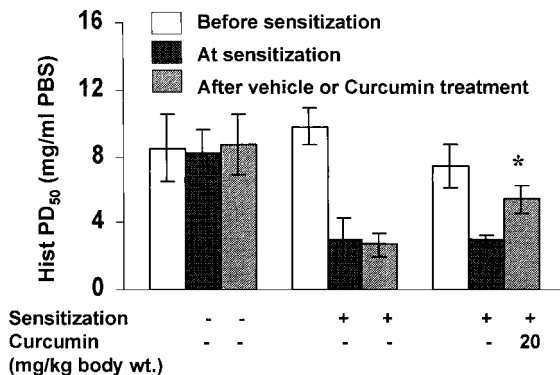


Fig. 4. Curcumin Decreases Airway Hyperreactivity to Histamine

Experiments were performed as described in Fig. 3 legend. After 24 h of OVA-inhalation, each individual guinea pig was housed in the body plethysmograph and Hist PD₅₀ values were determined as described in the Materials and Methods. Results are shown as the mean \pm S.E.M. ($n = 6$ in each). Students *t*-test was applied to determine the significant difference. * $p < 0.0043$ indicates the difference from vehicle treated sensitized control.

pigs (3.0 ± 1.3) as compared to normal guinea pigs (9.8 ± 1.1 , $p = 0.0002$). Treatment with curcumin (20 mg/kg body weight) significantly increased Hist PD₅₀ (*i.e.*, reduced the developed airway hyperreactivity to histamine). The mean value of Hist PD₅₀ increased upto 5.4 ± 0.8 as compared to the sensitized value, 3.0 ± 0.3 ($p < 0.0043$) (Fig. 4).

DISCUSSION

Asthma is an inflammatory airway disease where multiple pathways are involved in the pathogenesis and ultimately cause reversible airway constriction and airway hyperreactivity to a variety of stimuli. Experimental animal model of asthma is characterized by allergen-induced immediate airway constriction and late airway reactivity to a pharmacological vasoconstrictor such as histamine, methacholine *etc.* We demonstrate here that curcumin significantly reduced OVA-induced immediate airway constriction and late airway hyperreactivity to histamine in guinea pigs (Figs. 2–4).

The early airway response is mediated by IgE-dependent mast cell degranulation and resulting release of mediators such as histamine and leukotrienes. Curcumin is reported to

block histamine release by mast cells^{13,14} and inhibit lipoxygenase activity¹⁵ thereby possibly inhibit leukotrienes formation. Our observation of reduced airway constriction in guinea pigs could be attributed to these properties of curcumin.

Curcumin has also been demonstrated to inhibit production of inflammatory cytokines like IL-5¹⁶ and IL-8⁷ involved in the development of inflammation and also found to inhibit the activation of transcription factors like nuclear factor kappa-B (NF- κ B) and activating protein 1 (AP-1).¹⁷ The anti-adhesion property of curcumin was recently demonstrated.^{18,19} It is possible, therefore, that in the present study, curcumin might be intervening at one or some of the above mentioned pathways to reduce the asthmatic symptoms in guinea pigs. However, this remains to be determined in the future.

Although curcumin did not show full recovery of decreased SGaw and Hist PD₅₀, it showed significant improving effect on the impaired features of airways. However, curcumin is found not to be as effective as luteolin previously tested by us in a mouse model.²⁰ Nevertheless, the effective dose of curcumin (20 mg/kg body weight) was comparable to many of the anti-inflammatory compounds already tested in guinea pigs.^{21,22} Since curcumin is a natural product and has no side effects it could be considered as a non-steroidal anti-inflammatory molecule to develop anti-asthmatic drug.

In conclusion, our study demonstrates that curcumin significantly attenuates the impaired airway features in sensitized guinea pigs. However, further studies are required to understand the molecular mechanism of its anti-asthmatic action.

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