

Bronchodilatory Effect of Inhaled Neem oil in Guinea pigs

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Abstract

Objectives: Bitter taste receptors are recently found to be expressed in isolated human airway smooth muscle. Keeping this in view, the current study was aimed to evaluate the role of herbal bitter tastant, Neem oil, on histamine induced bronchoconstriction in guinea pigs.

Methods: Healthy adult guinea pigs of either sex were exposed to 1% histamine aerosol initially and after pre-treatment with distilled water (control), graded strengths of neem oil, the prototypic bitter tastant, quinine, a standard bronchodilator, salbutamol and a combination of salbutamol and neem oil, through inhalational route. The pre-drug and post-drug pre-convulsive time (PCT) was calculated, and percentage protection was found out using standard formula.

Results: Neem oil, in all the strengths used produced significant dose dependant protection from histamine induced bronchoconstriction equivalent to that seen with quinine and salbutamol. Combination of neem oil and salbutamol produced greater protection than the individual drugs.

Conclusions: The study results indicate that Neem oil has bronchodilatory effect probably by acting through bitter taste receptors.

Key Words: Bitter tastant, preconvulsive time, percentage protection.

Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are two chronic inflammatory lung diseases characterized by constriction of bronchial smooth muscle and air-flow limitation. The worldwide incidences of both the diseases are increasing day by day. Constriction of bronchial smooth muscle is the cause of morbidity and mortality in these diseases.¹ Corticosteroids and bronchodilators are the first-line therapies for their pharmacological management. Although β_2 agonists are the bronchodilators of choice, therapy with these agents are limited by development of

tachyphylaxis.² In spite of treatment, more than 50% of all these patients are not well controlled by currently available drugs. Hence an unmet need exists for additional efficacious therapies in the treatment of obstructive lung diseases such as asthma.

G-Protein Coupled Receptors (GPCRs) are the major receptor signalling family that regulate contraction and relaxation of ASM.³ By pan-GPCR screening; several bitter taste receptors are recently isolated from human ASM.^{4,5,6} Bitter taste receptors of tongue helps to avoid ingestion of environmental toxins. Similarly bitter taste receptors of lung might have evolved to escape from inhalation of environmental toxins.⁷ But in a recent study, it was observed that bitter taste receptor (TAS2R) agonists such as chloroquine, quinine, denatonium relax ASM through opening of BKca channels due to ASM membrane hyperpolarization.⁸ Inhalation of bitter tastant in an asthma mouse model was also found to decrease airway obstruction.⁸ Therefore, in the context of the need for efficacious bronchodilators to treat obstructive lung diseases, these receptors can serve as potential novel drug targets as there are thousands of known synthetic and naturally occurring ligands to these receptors (bitter tastant).

Herbal drugs constitute a major share of all the officially recognised alternate systems of medicine practiced in India and more than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine due to their evidence-based safety⁹. Neem oil, an herbal bitter tastant, acts through the bitter receptor and might act on these receptors located in the lungs. With this background this study was undertaken to assess the effect of bitter tastant, Neem oil, on histamine induced bronchoconstriction in guinea pigs and compare it with standard drugs.

Aims & Objectives

To evaluate the effect of inhaled Neem oil on histamine induced bronchoconstriction in guinea pigs.

Materials and methods

For this study, forty two healthy adult Guinea pigs of either sex, weighing between 300 to 600 g, were selected. They were divided into 7 equal groups of 6 animals in each, and kept in the Central Animal House under 12:12 day/night cycle, at an ambient temperature of $27 \pm 5^\circ\text{C}$. They were fed with standard diet in sufficient quantity and water was given ad libitum. The study was conducted in the Dept. of Pharmacology, V.S.S. Medical College, Burla, Sambalpur from April to September 2014 and its Animal Institutional Ethics Committee (IAEC) approved the protocol of the study. The animals were handled with due care as per CPCSEA guidelines.

After overnight fasting the animals were exposed to 1% Histamine acid phosphate inhalation through nebulizer in Histamine Chamber (Techno). The Pre-Convulsive Time (PCT) was calculated in each animal from the time they were exposed to histamine inhalation to the time of onset of convulsion due to asphyxia caused by bronchoconstriction¹⁰. As soon as the PCT was noted the animal was removed from the chamber and placed in fresh air. This was their Pre-drug PCT value i.e. T1. After 24 hrs, the overnight fasted guinea pigs were administered with aerosolised test and control drugs through inhalation in the histamine chamber as mentioned in the box below. Fifteen minutes later these animals were again exposed to 1% Histamine inhalation. The Post-drug PCT i.e. T2 was calculated. The values were expressed as Mean \pm SEM.

| Group | Drug administered | Dose |
|-------|--------------------------------|------------|
| 1 | Distilled water (Control) | 1ml |
| 2 | Neem oil | 5% |
| 3 | Neem oil | 10% |
| 4 | Neem oil | 20% |
| 5 | Quinine | 30% |
| 6 | Salbutamol Respirator solution | 0.1% |
| 7 | Salbutamol + Neem oil | 0.1% + 20% |

All the doses were given through inhalational route. Neem oil (100%) was obtained from Baidyanath Pharmaceuticals, Bhubaneswar, and emulsion was prepared in Pharmacy Lab. of V.S.S. Medical College by Continental (Dry Gum or 4:2:1) method. Salbutamol was obtained from CIPLA, Mumbai, and quinine from Shreya Life Science Pvt Ltd, Mumbai and Histamine from Himedia Pvt Ltd, Mumbai.

The protection offered by treatment was calculated by using the following formula: Percentage Protection = $(1 -$

$T1/T2) \times 100$, where T1 was the mean PCT before administration of drug and T2 was the mean PCT after administration of drug.

Statistical Analysis:

The post-drug (T2) values of each group were compared with their pre-drug (T1) values statistically by paired samples T- test and the post-drug (T2) values were compared among the groups by ANOVA followed by Post hoc Tukey test. All the values were analysed statistically using SPSS version 19.

RESULTS

The mean pre-drug and post-drug PCT values, and mean percentage protection produced by test and control drugs are depicted in table 1.

Table 1: Effect of drugs on pre-convulsive time (PCT) and percentage protection in histamine induced bronchoconstriction model in guinea pigs.

| Group | Drug | Dose (%) | Mean PCT (sec) \pm SEM | | % Protection |
|-------|-----------------------|----------|--------------------------|--|------------------|
| | | | Pre-Drug (T1) | Post-Drug (T2) | |
| 1 | Dist. water | 1ml | 60.17 \pm 2.64 | 60.33 \pm 2.49 ^{sa} | 02.72 \pm 2.71 |
| 2 | Neem oil | 5 | 60.00 \pm 2.59 | 80.5 \pm 2.43 ^{**a #b} | 25.13 \pm 3.86 |
| 3 | Neem oil | 10 | 59.83 \pm 0.54 | 100.33 \pm 2.84 ^{**a #c} | 40.14 \pm 1.65 |
| 4 | Neem oil | 20 | 56.83 \pm 2.51 | 129.33 \pm 8.76 ^{**a #d} | 55.47 \pm 2.42 |
| 5 | Quinine | 30 | 59.00 \pm 0.86 | 129.83 \pm 7.22 ^{**a #d, \$e} | 53.94 \pm 2.28 |
| 6 | Salbutamol | 0.1 | 59.83 \pm 1.83 | 179.17 \pm 5.50 ^{**a #f} | 66.58 \pm 0.60 |
| 7 | Salbutamol + Neem oil | 0.1+ 20 | 60.50 \pm 1.40 | 273.00 \pm 14.73 ^{**a #g} | 77.67 \pm 0.64 |

All values are expressed as mean \pm S.E.M. (n=6). ^{a-} Comparisons are made with Pre-Drug (T1) value (Paired Samples T-test). ^{b, c, d, e, f, g-} Comparisons of the post-drug (T2) values among the 7 groups are made by ANOVA followed by Tukey test. (^{b-} Comparisons are made with Group1, ^{c-} Comparisons are made with Group 1&2, ^{d-} Comparisons are made with Group 1, 2 & 3, ^{e-} Comparisons are made with Group 4, ^{f-} Comparisons are made with Group 1,2,3,4&5, ^{g-} Comparisons are made with Group 1,2,3,4,5&6). * $P < 0.05$, ** $P < 0.01$, # $P < 0.05$. \$ $P > 0.05$.

Distilled water produced no effect in the PCT in animals of Group 1. Pre-treatment with neem oil inhalation produced

significant increase in the post-drug PCT values, over the pre-drug values, starting from the strength of 5% and continuing up to 20%. This bronchodilatory effect of neem oil was dose dependant. Quinine 30% inhalation also produced protection from histamine induced bronchoconstriction, similar to that seen with neem oil (20%) and salbutamol (0.1%) inhalation. Addition of neem oil (20%) to salbutamol 0.1% produced greater protection than salbutamol alone. None of the animals showed any signs of toxicity and there was no death during experiment.

Discussion

Herbal drugs are used for the treatment of various common ailments as a part of traditional system of medicine, due to their potential efficacy and lack of adverse effects in contrast to their synthetic counterparts. Scientific validation of their pharmacological activities needs to be elaborately elucidated to establish their clinical efficacy so that they can be authenticated for use in modern medicine. Newer targets for action of drugs need to be discovered rather than synthesis of more and more me-too drugs.

Bitter taste receptors were recently found to be expressed on the human airway smooth muscle, predominantly of the TAS2R family.⁴ They comprise a new group of GPCRs that regulate airway calibre through PLC β -Ca⁺⁺ pathway. This signal leads to activation of large conductance Ca⁺ activated K⁺ channel and marked relaxation of airway smooth muscle in vivo and ex vivo^{2,4,7}.

Our study aimed to evaluate one such agonist of bitter receptors of herbal origin i.e. neem oil on histamine induced bronchoconstriction in guinea pigs. This model is most widely used to test the bronchodilatory effect of potential drugs.

The results of this study reveal that inhalation of neem oil can evoke significant relaxation of airway smooth muscle similar to that seen with inhalation of prototypic bitter tastant, quinine and the prototypical bronchodilator, salbutamol. Other workers have observed similar effect in isolated airway smooth muscle with quinine and chloroquine⁸.

Conclusion & Limitations

Neem oil inhalation can be potentially useful as a novel direct bronchodilator with clinical efficacy in obstructive lung diseases. However, clinical trials with the active principles of neem (e.g. azadirachtin) need to be undertaken in vitro and in-vivo studies to establish its use for therapeutic purposes.

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Biographies

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