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Short communication

Preliminary studies on the effect of rebamipide against the trypsin and egg-albumin induced experimental model of asthma

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The present investigation was carried out to study the effect of rebamipide in experimentally induced bronchial asthma in mice. Trypsin and egg-albumin induced chronic model of asthma was used and various parameters were measured on the 35th day. The asthmatic control group showed lower level of haemoglobin saturation with oxygen, tidal volume, airflow rate and higher respiratory rate, serum bicarbonate level, eosinophil count in bronchoalveolar lavage fluid and histamine level compared to the normal control group. Dexamethasone and rebamipide treated groups showed the return of all the above parameters towards normal values. Histopathological examination of lungs showed more prominent alveolar and muscular layer destruction in the asthmatic control group than in dexamethasone and rebamipide treated groups. Rebamipide showed a beneficial effect and might be used for the treatment of bronchial asthma.

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Asthma is a chronic inflammatory disease of the airways in which many cell types play a role, in particular mast cells, eosinophils and T lymphocytes. In susceptible individuals, inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or early morning. Inflammation causes an associated increase in airway responsiveness to a variety of stimuli (1). Recruitment of peripheral blood cells into inflamed airways is the result of adhesive interactions between circulating inflammatory and microvascular endothelial cells *via* the production of proinflammatory mediators, cytokines and chemokines, and the expression of cell surface adhesion molecules (2).

Rebamipide, an amino acid derivative of 2(1*H*)-quinolinone (Fig. 1), is used for mucosal protection, healing of gastroduodenal ulcers, and treatment of gastritis, colitis and inflammatory bowel disease (3–5). Rebamipide inhibits the NF-κB, expression of various adhesion molecules as well as the production of various inflammatory mediators (IL-1α,

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IL-8, TNF-α, histamine and leukotrienes) (6, 7). It inhibits mobilization of granulocytes, macrophages and neutrophils and production of IgG/IgM antibodies (8). In light of the above facts, the present investigation was carried out to study the potential effect of rebamipide in the trypsin and egg-albumin induced experimental model of asthma in mice.

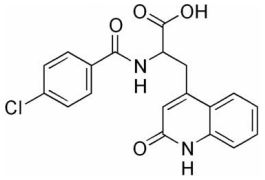

Fig. 1. Chemical structure of rebamipide.

EXPERIMENTAL

Chemicals

Rebamipide was obtained from Macleod Pharmaceuticals, India. Dexamethasone was obtained from Suvik Pharmaceutical Private Limited, India. Trypsin and egg-albumin were purchased from Rakesh Chemicals, India. Histamine hydrochloride was procured from Sigma Chemicals, USA. Saline (0.9 %, *m/V*, NaCl) and phosphate buffered saline (pH = 7.2) were used.

Animals

Healthy albino mice of either sex (n = 24), weighing 25–30 g were procured from Zydus Research Centre, India. The animals were housed at 25 ± 1 °C, 50 ± 15 % RH for 12 hour light-dark cycles, in polypropylene cages with free access to food and water *ad libitum*. The experimental protocol was approved by the Institutional (K. B. Institute of Pharmaceutical Education and Research) Animal Ethics Committee (IAEC) under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guideline, before carrying out the project.

Study design

A combination of trypsin and egg-albumin was used to induce asthmatic status in mice (9). The animals were divided into four groups of six animals each: group I – asthma, group II – asthma + rebamipide, group III – asthma + dexamethasone, group IV – normal control.

All animals (except group IV) were exposed to trypsin aerosol (1 mg mL^{-1}, 1 mL min^{-1}) once daily for 5 min, followed by a rest of 2 h and then exposed to egg-albumin aerosol (1 %, *m/V*, 1 mL min^{-1}) for 3 min. This procedure was repeated for 10 days and later egg-albumin aerosol was discontinued whereas trypsin exposure was continued until the 21st day. On the 21st day, after last exposure to trypsin, the animals were examined for the parameters mentioned below. Group I animals were exposed to trypsin and egg-albumin, but did not receive any drug treatment. They served as asthmatic control ani-

mals. Animals of group II received rebamipide (3 mg kg^{-1}, *p.o.*) and animals of group III received dexamethasone (5 mg kg^{-1}, *p.o.*) from day 22 to day 35. Group IV animals did not receive any treatment except saline and served as normal controls. On day 35, 2 h after of the last dose of treatment, only egg-albumin challenge was given.

On day 1 before any exposure (basal value), on day 21 after trypsin exposure and on day 35 after egg-albumin challenge, the following parameters were measured for each animal: pO_2, lung function (respiratory rate, air flow rate, tidal volume), blood (bicarbonate). On day 35, in addition to the above parameters, the following parameters were also measured: bronchoalveolar lavage (BAL), histamine in homogenate of lung tissue and histopathology of lung tissue.

Measurement of serum bicarbonate. – The method used in the present study to measure the serum bicarbonate level was slightly modified method described by Godkar (10).

Measurement of respiratory rate, airflow rate and tidal volume. – The measurement was done with the help of a respiratory volume transducer (11).

Differential leukocyte count in BAL fluid. – On the 35th day, 3 h after the egg-albumin challenge or just prior to animal death, whichever were earlier, the tracheobronchial tree was lavaged with 1 mL of saline 3 to 4 times. The fluid was collected and centrifuged at 2000 rpm for 5 min. The supernatant was discarded and the pellet was resuspended in 0.5 mL saline. A thin film of suspended saline was made on a clean grease-free slide and fixed with methyl alcohol for 3–5 min and dried. Then, a few drops of Geimsa stain in phosphate buffered saline (pH 6.8) were added and kept for 15 min or more. This was washed off with tap water and dried. The number of each type of leukocytes was determined under the microscope at 450× magnification.

Histamine release assay from lung tissues (12, 13) *and histopathology of lungs.* – On day 35, 3.5 h after the egg-albumin challenge or just prior to death of animals, the animals were sacrificed, lungs were dissected out and chopped into fragments. Chopped lung tissues were placed in tubes with 2 mL of ice-cold Ca^{2+}-free Tyrode solution and kept on ice until further use. Lung tissues (200 mg wet mass) were placed into test tubes. The test tubes were then supplemented with 1.8 mmol L^{-1} $CaCl_2$ and incubated for 10 min. at 37 °C. After that, the lung tissues were incubated with 2 mg L^{-1} egg-albumin for 15 min at 37 °C. After 15 min, the reaction was stopped by filtration of the medium through nylon mesh (100 μm). Histamine in the medium was determined fluorimetrically.

Dissected lungs were used for histopathological study.

Statistical analysis. – Experimental results were expressed as the mean ± SEM (n = 6). Statistical significance of the difference in parameters amongst groups was determined by one-way ANOVA followed by Tukey's multiple range test as well as by paired t-test.

RESULTS AND DISCUSSION

Serum bicarbonate

Challenging of animals with egg-albumin on day 35 of the study showed a significant ($p < 0.001$) higher serum bicarbonate level in the asthmatic control group compared

Table I. Effect of dexamethasone and rebamipide on serum bicarbonate level, respiratory rate, airflow rate and tidal volume

Parameter	Group I	Group II	Group III	Group IV
Serum bicarbonate level (mmol L^{-1})	48.83 ± 2.39	34.33 ± 1.17[a]	35.50 ± 0.56[a]	34.00 ± 1.24[b]
Respiratory rate (breaths per min)	193.33 ± 2.01	143.50 ± 7.79[c]	155.83 ± 3.07[c]	168.67 ± 4.66[c]
Air-flow rate (mL min^{-1})	0.96 ± 0.05	7.29 ± 0.55[c]	7.92 ± 0.41[c]	11.26 ± 0.32[c]
Tidal volume (mL)	0.02 ± 0.01	0.05 ± 0.02[c]	0.05 ± 0.01[c]	0.07 ± 0.02[c]

Values represent mean ± SEM of six animals in each group.
Group I – asthma, group II – dexamethasone (5 mg kg^{-1}), group III – rebamipide (3 mg kg^{-1}), group IV – normal control.
Significant difference *vs.* group I: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$.

to the normal control group, whereas significantly ($p < 0.05$) lower serum bicarbonate levels in dexamethasone and rebamipide-treated groups compared to the asthmatic control group (Table I).

Tidal volume, respiratory rate and airflow rate

Significantly ($p < 0.001$) lower tidal volume and air flow rate were observed in the asthmatic control group compared to the normal control group after egg-albumin challenge but there was a significant ($p < 0.001$) increase in those parameters in dexamethasone and rebamipide-treated animals. In contrast to the tidal volume and air flow rate, a significantly ($p < 0.001$) lower respiratory rate was observed in dexamethasone and rebamipide-treated groups compared to the asthmatic control group (Table I).

BAL fluid

Challenging of animals with trypsin and egg-albumin showed significantly ($p < 0.001$) higher eosinophil count in the asthmatic control group compared to the normal control

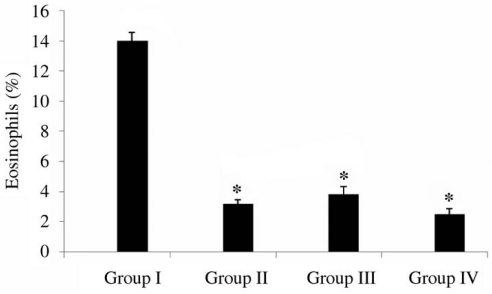

Fig. 2. Effect of dexamethasone and rebamipide on eosinophil count in BAL fluid. Values represent mean ± SEM of six animals in each group. Group I – asthma, group II – dexamethasone (5 mg kg^{-1}), group III – rebamipide (3 mg kg^{-1}), group IV – normal control. Significant difference *vs.* group I: *$p < 0.001$.

group on the 35th day of study. Also, there was a significant ($p < 0.001$) decrease in eosinophil count in the animals subjected to dexamethasone and rebamipide (Fig. 2).

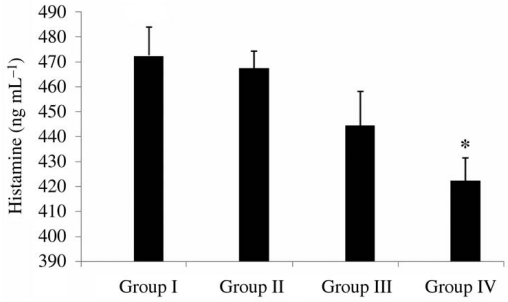

Fig. 3. Effect of dexamethasone and rebamipide on histamine concentration. Values represent mean ± SEM of six animals in each group. Group I – asthma, group II – dexamethasone (5 mg kg^{-1}), group III – rebamipide (3 mg kg^{-1}), group IV – normal control. Significant difference between groups I and IV: *$p < 0.001$.

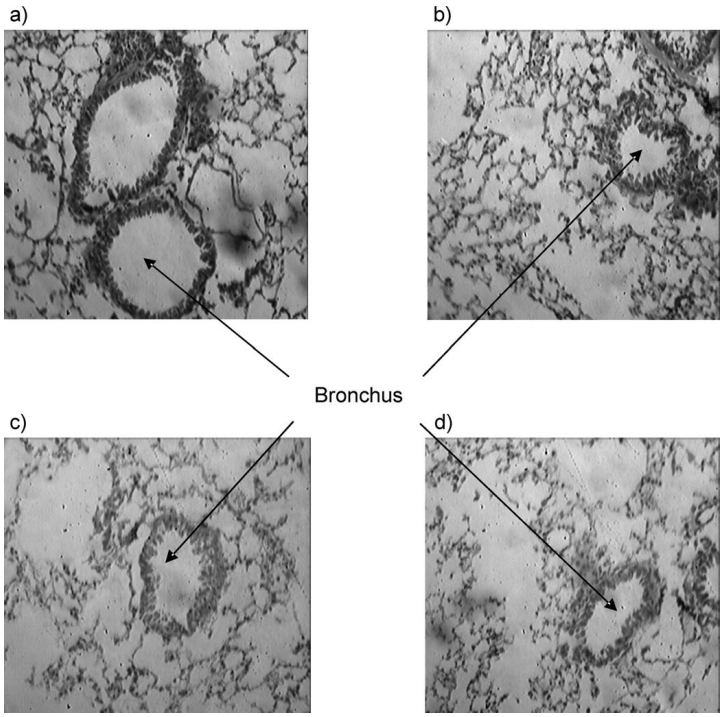

Fig. 4. Histopathology of lung: a) normal control mice, b) asthmatic mice, c) dexamethasone-treated mice, and d) rebamipide-treated mice (magnification 400×, hematoxylin stain).

Histamine release

Significantly ($p < 0.001$) higher histamine level was observed in the asthmatic control group compared to the normal control group, but in the animals subjected to rebamipide treatment, the histamine level decreased compared to the asthmatic control group (Fig. 3).

Histopathology of lungs

Normal control animals showed an intact bronchial structure (Fig. 4a), whereas trypsin and egg-albumin-sensitized animals showed marked inflammation and destruction of bronchial wall lining (Fig. 4b). Dexamethasone and rebamipide treated animals showed minimal destruction in bronchial wall compared to the asthmatic control group (Figs. 4c and 4d).

CONCLUSIONS

It can be concluded that rebamipide has beneficial effects in trypsin and egg-albumin induced experimental bronchial asthma and might be used for its treatment. Further clinical research of rebamipide will be necessary to support the present investigation.

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S A Ž E T A K

Preliminarno ispitivanje djelovanja rebamipida na model astme inducirane tripsinom i albuminom jajeta

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U radu je ispitivano djelovanje rebamipida na eksperimentalno induciranu bronhijalnu astmu u miševa. Astma uzrokovana tripsinom i albuminom jajeta model je kronične astme. Nakon 35. dana mjereni su različiti parametri. Kontrolna skupina s astmom imala je nižu koncentraciju oksihemoglobina, dišni volumen, protok zraka, koncentraciju SOD-a, a veći broj udisaja i izdisaja, koncentraciju bikarbonata u serumu, broj eozinofila u BAL fluidu i veću koncentraciju histamina u odnosu na kontrolnu skupinu bez astme. Skupina životinja tretirana deksametazonom i rebamipidom imala je veći pO_2, dišni volumen, protok zraka i koncentraciju SOD-a, a smanjenu respiraciju, koncentraciju bikarbonata, broj eozinofila u BAL tekućini i koncentraciju histamina u odnosu na kontrolnu skupinu s astmom. Histopatološka ispitivanja pluća pokazala su jače izražena oštećenja alveola i mišićnog sloja u kontrolnoj skupini s astmom nego u skupini tretiranoj deksametazonom i rebamipidom. Rebamipid je imao povoljan učinak na astmatičnu skupinu i mogao bi se upotrijebiti u terapiji bronhalne astme.

Ključne riječi: astma, rebamipid, tripsin, histamin

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