Summary and Conclusions
SUMMARY AND CONCLUSIONS

The vagal sensory receptors provide sensory inputs to the central nervous system about the changes that take place in the airways and lungs in various pathophysiological conditions to produce appropriate reflex responses. These receptors are considered to play an important role in the respiratory symptoms associated with various inflammatory lung diseases such as asthma. The present study provides an experimental basis for these claims.

In this investigation, an attempt has been made to determine the behavior of vagal sensory receptors mainly RARs and SARs during the oxidative stress induced airway hyperreactivity as observed in the case of asthma. The modulation of their activities by dietary antioxidants such as vitamin C and vitamin E has also been explored.

For making these observations, a guinea pig model of asthma was successfully developed using ovalbumin as an allergen. The animals were divided into six Groups. In each animal, pulmonary mechanics was determined and vagal afferent activity originating from RARs and SARs was recorded. The changes in pulmonary mechanics and vagal afferent activity to histamine inhalation were observed. Finally, the study was repeated after intake of the antioxidants, vitamin C and vitamin E.

- Group 1 served as control.
- In Group 2, the animals were sensitized with ovalbumin. It was divided into two Groups- Group 2a and Group 2b.
- In Group 2a, twenty eight days after sensitization, ovalbumin challenge was given and the immediate asthmatic response was investigated.
- In Group 2b, the animals were sensitized and challenged with ovalbumin and were utilized 24 hr after challenge to observe the response in late asthmatic phase.
- In Group 3, the effect of in vivo generation of oxidants by xanthine xanthine oxidase inhalation was determined.
• In Group 4a and Group 4b, the interventions were the same as in Group 2a and Group 2b respectively. In these Groups, the diets of the animals were supplemented with the antioxidants vitamin C and vitamin E.

The salient observations of the present study are summarized below.

**Group 1**

• In this Group, saline inhalation did not produce any significant change either in pulmonary mechanics or afferent activity.
• The ED$_{50}$ was achieved at the histamine dose of 0.32 mg/ml.
• At this dose, there was a significant rise in the tracheal pressure and airway resistance along with a significant fall in the dynamic compliance.
• Both the RARs and SARs were stimulated significantly at this dose.
• Histology showed no gross lung pathology.

**Group 2a**

• Following ovalbumin sensitization in Group 2a, the basal RAR activity alone was significantly higher as compared to that in Group 1.
• There was a significant rise in the basal tracheal pressure as compared to that in Group 1.
• Following ovalbumin challenge in this group, there were significant increases in tracheal pressure and airway resistance along with a significant fall in dynamic compliance.
• Along with the changes in pulmonary mechanics, ovalbumin inhalation stimulated the RARs and to a lesser extent SARs significantly.
• In this background, it was observed that the ED$_{50}$ histamine dose was significantly reduced in this Group compared to Group 1 thereby indicating airway hyperresponsiveness.
• At the ED$_{50}$ histamine dose, there was a significant increase in activity of RARs which was significantly higher compared to their response to ED$_{50}$ histamine dose in Group 1.
• At the ED$_{50}$ histamine dose, the SARs were also stimulated significantly. However, the response was similar to that for the ED$_{50}$ histamine dose in Group 1.
• There was a significant increase in the production of superoxide anion and lipid peroxidation products along with a significant fall in the levels of antioxidant enzyme in this Group compared to those in Group 1.
• Lung histology showed moderate infiltration of inflammatory cells such as neutrophils with monocytes, lymphocytes and macrophages along with mild to moderate edema and mild epithelial damage.

Conclusions

• The allergen challenge in the sensitized animals leads to widespread bronchoconstriction as evidenced by changes in pulmonary mechanics.
• These changes in airway mechanics were further associated with increases in the receptor activity, oxidative stress and infiltration of inflammatory cells along with fluid flux and epithelial damage.
• Following allergen challenge, there was an increase in the sensitivity of airways and airway receptors to histamine.
• Between the RARs and SARs, the responses of RARs were greater.

Group 2b

• In Group 2b, there was an increase in the basal tracheal pressure as compared to that in Group 1.
• The basal RAR activity was significantly higher in this Group as compared to that in Group 1.
• The ED$_{50}$ histamine dose was also reduced significantly compared to Group 1 thereby suggesting airway hyperresponsiveness.
• At the ED$_{50}$ dose of histamine, there was significant stimulation of RARs and it was significantly greater than their response to the ED$_{50}$ dose of histamine in Group 1.
• At the ED$_{50}$ dose of histamine, the SARs were also stimulated significantly and this stimulation was similar to their response to the ED$_{50}$ dose in Group 1.
Summary and Conclusions

- There was a significant increase in the production of superoxide anion and lipid peroxidation products along with a significant fall in the levels of antioxidant enzymes compared to those in Group 1.
- Lung histology showed high infiltration of inflammatory cells such as eosinophils with monocytes, lymphocytes and macrophages along with moderate level of edema and epithelial damage.

Conclusions

- The bronchoconstriction and increased RAR activity continued in late asthmatic phase also.
- In this phase, there was an increase in the airway responsiveness to histamine.
- There was an increase in the response of RARs and SARs to histamine. However, the responses of RARs were greater.
- These increases in airway sensitivity and afferent activity were further associated with oxidative stress and other inflammatory changes such as infiltration of inflammatory cells along with fluid flux and epithelial damage.

Group 3

- In Group 3, xanthine - xanthine oxidase inhalation resulted in increases in tracheal pressure and airway resistance and a decrease in dynamic compliance compared to those in Group 1.
- Following xanthine - xanthine oxidase inhalation, there was increase in the activity of RARs and SARs.
- The ED$_{50}$ for histamine was also reduced significantly in this Group, as compared to that in Group 1.
- At the ED$_{50}$ histamine dose, both the RARs and SARs were activated significantly to a similar extent as in Group 1.
- These changes in the airway sensitivity and receptor activity were associated with significant increases in the production of superoxide anion and lipid peroxidation products along with a significant fall in the levels of antioxidant enzymes as compared to those in Group 1.
• In the lung sections, there was mild infiltration of inflammatory cells such as neutrophils, monocytes, lymphocytes and macrophages along the peribronchiolar and perivascular region with mild edema and epithelial damage.

Conclusions

• The in vivo generation of oxidants led to bronchoconstriction as evidenced by changes in pulmonary mechanics.
• Along with these changes in airway mechanics, there were significant stimulations of both RARs and SARs.
• There was an increase in the airway responsiveness to histamine.
• The responses of RARs and SARs to histamine were increased.
• These changes in airway sensitivity and afferent activity were further associated with oxidative stress and mild infiltration of inflammatory cells along with mild fluid flux and epithelial damage.

Group 4a

• In Group 4a, there was a significant increase in the basal activity of RARs compared to that in Group 1. Though less, it was not significantly different from that in Group 2a.
• This increase was not associated with an increase in basal tracheal pressure (the basal tracheal pressure was similar to that in Group 1).
• There was no significant change in the basal activity of SARs.
• Following ovalbumin challenge, the tracheal pressure and airway resistance were increased.
• When compared to Group 2a, the changes in pulmonary mechanics were significantly reduced suggesting thereby that antioxidant intake might reduce the severity of the attack.
• Along with the changes in pulmonary mechanics following ovalbumin challenge, there was a significant increase in RAR activity.
• This response was significantly less compared to that in Group 2a but significantly greater than the response to saline inhalation in Group 1, suggesting that antioxidant intake reduced the responses of RARs also.
• The ED$_{50}$ dose of histamine was higher when compared to that in Group 2a but was similar to the ED$_{50}$ dose of histamine in Group 1.

• As observed in pulmonary mechanics, there was a reversal in the RAR response to histamine inhalation. The response was lower as compared to Group 2a but was similar to Group 1.

• The reversal in the response to histamine was noted in SARs also.

• Along with these changes, there was a reversal in oxidative stress parameters indicated by decreases in production of superoxide anion and lipid peroxidation products along with a significant rise in the levels of antioxidant enzymes compared to those in Group 2a.

• There were increases in the plasma levels of vitamin C and vitamin E.

• A reversal in pulmonary inflammation was also observed. In the lung sections, the infiltration of inflammatory cells was reduced along with less of epithelial damage.

Conclusions

• There was a reduction in the responsiveness of the airways and the responses of airway sensory receptors to ovalbumin challenge following antioxidant supplementation.

• There was also partial reversal in the responsiveness of the airways and the responses of the sensory receptors to histamine following antioxidant supplementation thereby suggesting that part of these responses could be oxidative stress mediated.

• The partial reversal was also evident in oxidative stress parameters and pulmonary inflammation with a reduction in the infiltration of inflammatory cells, fluid flux and epithelial damage.

Group 4b

• In Group 4b, the pulmonary mechanics in the basal state was similar to that in Group 1.

• Unlike the observations in Group 2b, the basal RAR activity in this Group was not significantly different from that in Group 1.
Summary and Conclusions

- The ED$_{50}$ histamine dose was significantly higher when compared to that in Group 2b. However, it was similar to that in Group 1.
- As observed in the case in Group 4a, in this Group also, the ED$_{50}$ histamine dose responses of airways was similar to the ED$_{50}$ histamine dose responses observed in Group 1.
- At the ED$_{50}$ histamine dose, the RARs were stimulated significantly. However, their response was significantly lower when compared with that in Group 2b. The responses were similar to those elicited in Group 1.
- At the ED$_{50}$ dose, the SARs were also stimulated. The response was similar to that in Group 1.
- There was reversal in oxidative stress parameters indicated by significant decreases in production of superoxide anion and lipid peroxidation products along with a significant rise in the levels of antioxidant enzymes when compared to these in Group 2b.
- A reversal in pulmonary inflammation was also observed with a reduction in the infiltration of eosinophils, edema and epithelial damage.

Conclusions

- In this group also, there was partial reversal in the responsiveness of the airway and lung receptors to histamine following antioxidant supplementation thereby suggesting that part of these responses could be oxidative stress mediated.
- The partial reversal was also evident in oxidative stress parameters and pulmonary inflammation.

From the present study, it may be concluded that oxidative stress plays a significant role in inducing the airway hyperresponsiveness in asthma. Upon exposure to the allergen, there occurs widespread bronchoconstriction resulting in increases in tracheal pressure and airway resistance and decrease in dynamic compliance. All these changes lead to the stimulation of the vagal sensory receptors connected to myelinated afferents, the RARs and SARs. The RARs were more sensitive to the allergen than the SARs. Exposure to an allergen increases the sensitivity of airways and lung receptors to lung autacoids such as histamine. Since an increase in tracheal tone has been reported
following RAR stimulation (Kappagoda et al., 1989), it is proposed that their activation would lead to further bronchoconstriction. Their stimulation increases mucus production (Yu et al., 1989) and cause cough. All these symptoms worsen asthma. Though less sensitive, the SARs are also stimulated. The SARs may counteract the bronchoconstriction as their activation causes bronchodilation (Paintal, 1973). During the late asthmatic response, there is an increase in the sensitivity of airways and vagal sensory receptors to lung autacoids. In both early and late asthmatic phases there is fluid flux and infiltration of inflammatory cells in the lungs. These changes are associated with increased oxidative stress parameters. All these changes stimulate the RARs (Ravi and Kappagoda, 1990; Ravi et al. 1989; Bonham et al., 1996). Thus, RAR activation may give rise to the dyspneic sensation associated with asthma.

Upon supplementation with dietary antioxidants, there is a partial reversal in the airway hyperresponsiveness and inflammation. Also the sensitivity of these vagal sensory receptors to histamine is also reversed partially. These findings indicate that oxidative stress plays a significant role in inducing the airway hyperresponsiveness in asthma. The in vivo generation of oxidants by xanthine-xanthine oxidase inhalation causes the bronchoconstriction and stimulation of these receptors. The study provides direct evidence that oxidants activate lung receptors. Even though the results are promising, since allergic disorders such as asthma are multifactorial, blocking oxidative stress alone is unlikely to lead to complete resolution of bronchoconstriction, inflammation and receptor activation but such an approach may prove to be a useful adjunct therapy.