

## BACKGROUND:

Asthma is a chronic inflammatory disorder of the airways in which a number of cells and cellular elements play a role. It is a lifetime disease which is not completely curable. Many factors contribute to the pathogenesis of asthma, oxidative stress being an important one. In healthy humans, the toxicity of free radicals is inhibited by enzymatic and non-enzymatic systems. In asthmatics, the function of these endogenous systems is impaired. Despite treatment, asthma morbidity and mortality steadily increases. The search for new drugs can be based on the knowledge of the pathological changes associated with the disease, and can also be inspired by folk medicine. As regards the latter approach, new, more potent substances could be obtained by isolation and chemical modification of natural products. The aim of this doctoral thesis was to elucidate the effects of selected substances on the respiratory system of rats by *in vivo* and *in vitro* methods. We investigated the effects of orally administered  $\alpha$ -tocopherol on the lung response in an *in vivo* rat model of allergic asthma. The second part of this thesis deals with natural quinazoline alkaloids (*Justicia adhatoda* standardized extract, vasicine, vasicinone) and their synthetic analogues, and their relaxant effects on an isolated rat trachea. The relaxant effects of these compounds were compared with theophylline, a standard drug used in asthma treatment.

## METHOD:

➤ *In vivo* study: classical experimental rat model of allergic asthma was used. All animals were actively sensitized with ovalbumin OA (1 mg) and aluminium hydroxide (100 mg) suspended in saline solution (1 ml) intraperitoneally injected into each rat. The injection was repeated for three consecutive days. Sensitized animals were used in the experiments on the 21st day after sensitization. In the meantime, the rats were treated with  $\alpha$ -tocopherol (400 mg/kg/day – treated group) or the vehicle (positive control group, negative control group) for 10 days. One hour after the last  $\alpha$ -tocopherol or vehicle administration, the animals were exposed to ovalbumin aerosol (100 mg per 2 ml of saline). The sham group (negative control group) was challenged with saline. After 1 or 24 h from the antigen challenge, the animals were anaesthetized with pentobarbital (45 mg/kg i.p.). A heparinized catheter was inserted into the right jugular vein for i.v. drug administration. After the tracheostomy, the trachea was cannulated and connected to a Hugo Sachs ventilator (90 breaths/min, 10 ml/kg). The animals were premedicated by an i.v. succinylcholine and propranolol injection and the bronchial

responsiveness to cumulative i.v. 5-HT administration (5–50 µg/kg) was assessed. After the measurement of the lung responsiveness, the rats were sacrificed with an overdose of pentobarbital. The lungs were removed and bronchoalveolar lavage fluid (BALF) was pooled in order to evaluate total and differential cell count. Histopathological examination of the rat lungs was done.

➤ *In vitro* study: to verify the relaxant activity of the tested substances, the classical experimental model of isolated rat trachea was used. The isolated trachea was cut into two rings. Each ring was then cut opened opposite to the tracheal smooth muscle and mounted under initial tension of 1 g in the Schuler Organ Bath in such a way that free relaxation or contraction of the smooth muscle was possible. The relaxation or contraction of the tracheal smooth muscle was registered by an isometric transducer and recorded (Ugo Basile, Italy). Adequate conditions were guaranteed by the Krebs-Henseleit solution maintained under constant temperature of 37 °C. The tissue specific holder includes an integrated frit at the back of the holder which allows carbogene (a gas mixture containing 5 % CO<sub>2</sub> and 95 % O<sub>2</sub>) to pass continuously through the Krebs-Henseleit solution. The trachea was precontracted by carbachol (10<sup>-5</sup> M). The relaxant effects of eight cumulative concentrations of each tested compound were examined. The maximal relaxation was induced by theophylline (3×10<sup>-3</sup> M), which was also used as a positive control. A decrease in the tone of the tracheal smooth muscle was considered as a relaxant effect and was expressed as a percentage of change in proportion to the maximum relaxation. The ED<sub>50</sub> of each tested compound, which is defined as the concentration producing 50 % relaxation of the precontracted trachea by the used agonist, was calculated using the non-linear regression method (GraphPad Prism).

## RESULTS:

➤ *In vivo* method: one hour after allergen challenge, i.v. serotonin administration induced a dose-related bronchoconstrictor response in all rats. No differences in the bronchospasm were found among the groups. Within 24 h after antigen challenge, i.v. cumulative administration of serotonin induced a dose-dependent and significant increase in overflow volume in the positive control group compared with the negative control group. In tocopherol premedicated animals, no significant changes were found. A tendency to an antihyperresponsiveness effect was observed in comparison with the positive control. In the BALF collected one hour after saline or ovalbumin exposure, no differences in the total cell

count were found among the groups. Similarly, no changes were found in the differential cell count. Twenty-four hours after antigen exposure, the positive control group showed a significantly higher total number of cells in the BALF compared with the negative control. The increase in the total cell number was not significantly changed by tocopherol, even though there was a tendency towards lower values. The differential cell count showed an increase in eosinophils and a significant increase in neutrophils in the positive control group BALF compared to the negative control. The increased percentage of eosinophils and neutrophils showed a diminishing trend in the tocopherol-treated rats but the difference was insignificant. The histopathological examination of both positive control and tocopherol-treated animals showed inflammatory changes compared with negative control. In the treated animals, no differences in quantitative and qualitative parameters were found in comparison with the positive group.

➤ *In vitro* method: we found out that all tested natural quinazoline alkaloids (*Justicia adhatoda* standardized extract, vasicine, vasicinone) had a relaxant activity on isolated rat trachea. The standardized extract and vasicine had a significantly higher relaxant effect than theophylline. Vasicinone showed a significantly lower relaxant activity than the standard. Subsequent evaluation of the relaxant effects of simple derivatives of quinazolin-4-ol showed that the structures from this group were not very potent. None of these four tested derivatives had a significantly higher activity in comparison to theophylline. The second synthetic group tested (quinazoline derivatives with an ethylamine fragment) was more successful – two out of the three tested compounds were significantly more efficient than theophylline. The derivatives of the last group tested (quinazoline derivatives bearing ethoxy, ethylsulfanyl and propoxy fragments) were the most potent ones. All five tested derivatives had a significantly higher relaxant activity than the standard.

#### CONCLUSION:

➤ Based on *in vivo* experiments, we can conclude that orally administered  $\alpha$ -tocopherol did not significantly influence bronchial hyperresponsiveness or inflammatory cells infiltration. Histopathological analysis revealed that the inflammation and the hypertrophy of the smooth muscle occurred in both groups, with no differences having been found. The airway

hyperresponsiveness to 5-HT observed in antigen-challenged sensitized rats tended to decrease after the administration of  $\alpha$ -tocopherol. After  $\alpha$ -tocopherol administration, a decreasing tendency in neutrophil and eosinophil numbers was observed in comparison to positive control. Even though these changes were statistically insignificant, a possible therapeutical potential of  $\alpha$ -tocopherol should not be ruled out.

➤ Based on *in vitro* experiments, we can conclude that all tested natural quinazoline alkaloids and most of the synthetic derivatives had a relaxant effect on isolated rat trachea. The most successful derivatives of the tested substances were synthetic quinazoline derivatives with ethyloxy, ethylamine and propyloxy fragments.