ARTICLE — EXPERIMENTAL STUDY

The influence of inhaled furosemide on adverse effects of ACE-inhibitors in airways

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Abstract

Background: **A persistent, chronic dry cough is the most common adverse effect of angiotensin converting enzyme (ACE) inhibitors therapy. The mechanism of this respiratory adverse effect is related to the inhibition of ACE and the accumulation of bradykinin, substance P, prostanoids and inflammatory neuropeptides in the airways.**
Main purpose: **The aim of this study was to follow the relationship between 15-days administration of enalapril and the defence reflexes of the airways of experimental animals and possibility of pharmacological restriction with inhaled furosemide.**
Methods: **From the defence reflexes of the airways the changes of the parameters of a mechanically induced cough in nonanaesthetized cats were measured. The reactivity of the smooth muscle of the airways to the bronchoconstrictor mediator was evaluated by in vitro method. The enalapril was administered for 15-days in the dosage of 5 mg/kg b.w. p.o., inhaled furosemide for 15-days in the dosage 10 mg/kg b.w.**
Results: **The results suggested that long-lasting administration of enalapril resulted in a significant increase of measured cough parameters and increased reactivity of tracheal smooth muscle to the histamine. The reactivity of the lung smooth muscle was not influenced significantly after enalapril treatment. Inhaled furosemide administered with enalapril significantly decreased the enalapril induced cough and decreased enalapril potentiated reactivity of the tracheal smooth muscle to the histamine.**
Conclusion: **The results showed the protective effect of inhaled furosemide against the respiratory adverse effects induced by ACE-inhibitors administration.** ***(Fig. 5, Ref. 20.)***
Key words: cough, bronchoconstriction, enalapril, furosemide.

The inhibitors of angiotensin-converting enzyme (ACE) are the drugs of choice for the treatment of hypertension and congestive heart disease. However, it has been reported that in some of patients ACE-inhibitors induce a dry, non-productive cough, with a frequency of occurrence between 0.2—37 %. Other airway reactions following ACE-inhibitor therapy, dyspnoe and wheezing, occur less frequently (Israili and Hall, 1992).

The therapeutic effectiveness of this pharmacological group is connected to the inhibition of enzyme angiotensin-convertase (kininase II), which plays a pivotal role in regulation of renin—angiotensin and kinin—kallikreine system. The mechanism of the respiratory adverse effects is unrelated to the inhibition of renin—angiotensin system, since treatment with angiotensin receptor blockers did not cause similar problems (Goldberg et al., 1996; Benz et al., 1997). The negative effects of ACE-inhibitors on defence reflexes of the airways are a link with the metabolism of kinins and tachykinins and consequent accumulation of bradykinin (Fox et al., 1996 a), substance P, neurokinin A and B in the respiratory tract (Sekizawa et al., 1996). Additional factors, which enhanced the respiratory side effects of ACE-inhibitors are the stimulation of phospholipase A_2 and the induction of mastocyte degranulation by bradykinine and substance P (Trifillief et al., 1993).

The above-mentioned pro-inflammatory mediators (bradykinin, substance-P, histamine, prostaglandins), whose level shows the increase in the airways after treatment with ACE-inhibitors, stimulate the rapidly adapting receptors myelinated vagal fibres (Hargreaves et al., 1992) and unmyelinated C-fibres (Fox, 1996 b), and consequently provoke the cough reflex.

In addition to their effect to the cough receptors, these substances could be capable to increasing the reactivity of the airway smooth muscle (Trifilieff at al., 1993).

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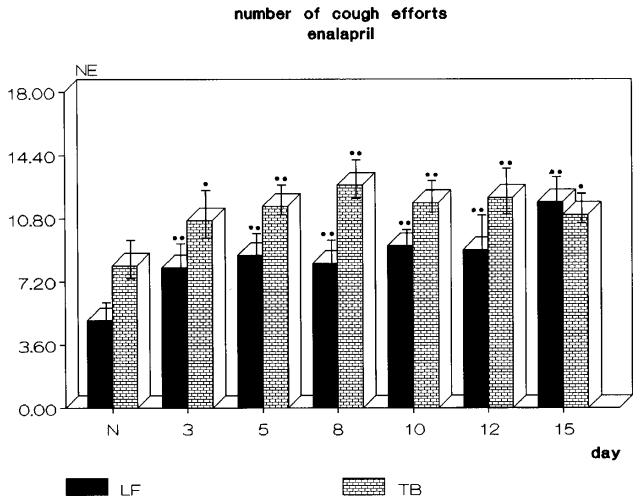


Fig. 1. Changes in the number of cough efforts (NE) from laryngopharyngeal (LF) and tracheobronchial (TB) area of the airways of the non-anaesthetized cats, before enalapril administration (N) and during 15-days enalapril administration. The columns represent average values, the dispersion is mean error of average. Statistical significance was calculated according to Wilcoxon-Wilcox test ($^{*}p<0.05$, $^{**}p<0.01$).

Clinical trials and experimental studies dealing with ACE-inhibitors treatment have been currently aimed at managing the cough induced by applying the above mentioned group of substances. According to our opinion, it is necessary to begin with a detailed knowledge of pathomechanism of the origin of the afore-mentioned adverse respiratory effects in order to select the substances capable to minimize or eliminate the side effects of the ACE-inhibitors in the respiratory tract. The basic condition, that should be kept if the cough is to be eliminated by means of the pharmacological intervention, consists in maintaining the primary pharmacological efficacy of ACE-inhibitors, thanks to which they are so widely used in clinical practice.

The aim of this study, was to follow the effect of the long-lasting administration of the enalapril on the defence reflexes of the airways. The first defence reflex followed was the sensitivity of the cough reflex induced by the mechanical irritation of the laryngopharyngeal and tracheobronchial mucous membrane in the non-anaesthetized cats. The second one followed was the reactivity of the tracheal and lung smooth muscle to the histamine by in vitro method. The following phase of the study was aimed at monitoring of the possible effect of inhaled furosemide on the respiratory adverse effects of the enalapril.

Material and methods

Mechanically induced cough by in vivo method

A method of mechanical stimulation of the laryngopharyngeal and tracheobronchial area of the airways of non-anaesthetized cats of both sexes weighing 1500—2500 g was used in the experiment (Nosáľová et al., 1993). After several days of quarantine, a tracheal cannula was surgically implanted into the animals, which served for the mechanical stimulation of the airways with nylon fibre 0.35 mm in diameter, as well as for recording the side tracheal pressure. The number of cough efforts (NE) was evaluated on the basis of the pressure values recorded during the experiment from both laryngopharyngeal (LF) and tracheobronchial (TB) area of the airways. The values of the cough parameters measured before the application of the substances, represented the control values (N). Enalapril was administered perorally as a saline solution in the dose 5 mg/kg b.w. to the first group of experimental animals for 15 days. The second group of animals was treated for 15 days with enalapril (dose 5 mg/kg/day perorally) and furosemide (dose 10 mg/kg/day by inhalation). The effect of enalapril and simultaneous administration of enalapril with furosemide on the cough parameters was monitored in the intervals 3, 5, 8, 10, 12, 15 days.

The results of this experiment were evaluated by Wilcoxon and Wilcox (1964) method.

Reactivity of the smooth muscle of the airways by the in vitro method

The reactivity of the tracheal and lung smooth muscle was estimated by the in vitro method (Strapková et al., 1997), after 15 days administration of enalapril (5 mg/kg/day) and the combined administration of enalapril (5 mg/kg/day) with furosemide (10 mg/kg/day).

The preparation of cat tracheal and lung strips were placed in 20-ml organ chamber containing Krebs-Henseleit buffer of the following composition (M): NaCl, 110.0 KCl, 4.8 $CaCl_2$, 2.35 $MgSO_4$, 1.20 $KHPO_4$, 1.20 $NaHCO_3$, 25.0 in glass-distilled water. Organ chambers were maintained at 36.5±0.5 °C and were aerated continuously with mixture 95 % O_2 and 5 % CO_2, to maintain pH 7.5±0.1. The tissue strips were initially set to 4 g of tension (30 minutes-loading phase). After this period, the tension in each tissue segment was readjusted to a baseline of 2 g (30 minutes adaptation phase). During these periods the tissue strip was washed at 15-min. intervals. The amplitude of contraction (mN) of the tracheal and lung smooth muscle to the cumulative doses of histamine (10^{-8}—10^{-3} mol.l^{-1}) was used for the evaluation of the reactivity.

For the statistical analysis Student's *t*-test for unpaired data was used.

Results

During 15-days enalapril administration (5 mg/kg/day) the sensitivity of the cough reflex was investigated by the method of a mechanical stimulation of the airways in non-anaesthetized cats. Based on the results, in 3, 5, 8, 10, 12, 15 day of enalapril administration it was found that a statistically significant increase of the number of cough efforts (NE) occured from laryngopharyngeal and tracheobronchial mucous membrane of the airways (Fig. 1).

Simultaneous 15-day applications of the enalapril with inhaled furosemide (10 mg/kg/day), in comparison to enalapril monotherapy, resulted in the significant decrease of the number of the cough from the laryngopharyngeal and tracheobronchial area of the airways (Fig. 2).

Figure 3 compares the changes in the number of the cough efforts from laryngopharyngeal and tracheobronchial region of the airways, during 15-days enalapril monotherapy, and during combined therapy enalapril with inhaled furosemide.

The potentiation of the cough parameters with enalapril administration and its following suppression with inhaled furosemide, served as motivation for monitoring of the reactivity of the airways

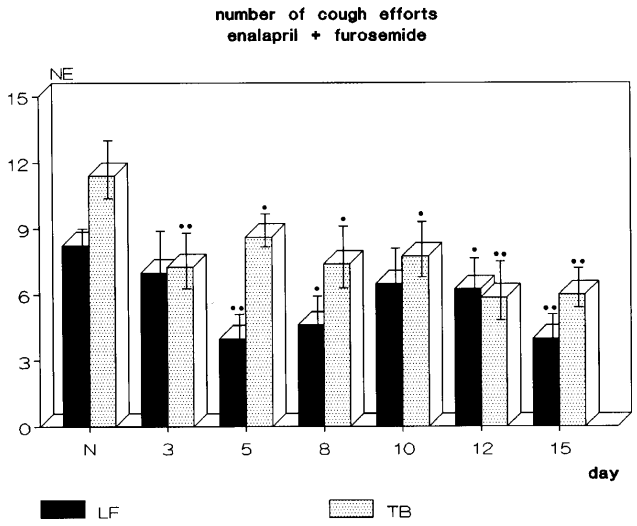


Fig. 2. Changes in the number of cough efforts (NE) from laryngopharyngeal (LF) and tracheobronchial (TB) area of the airways, before drug administration (N) and during 15-days administration of enalapril+inhaled furosemide.

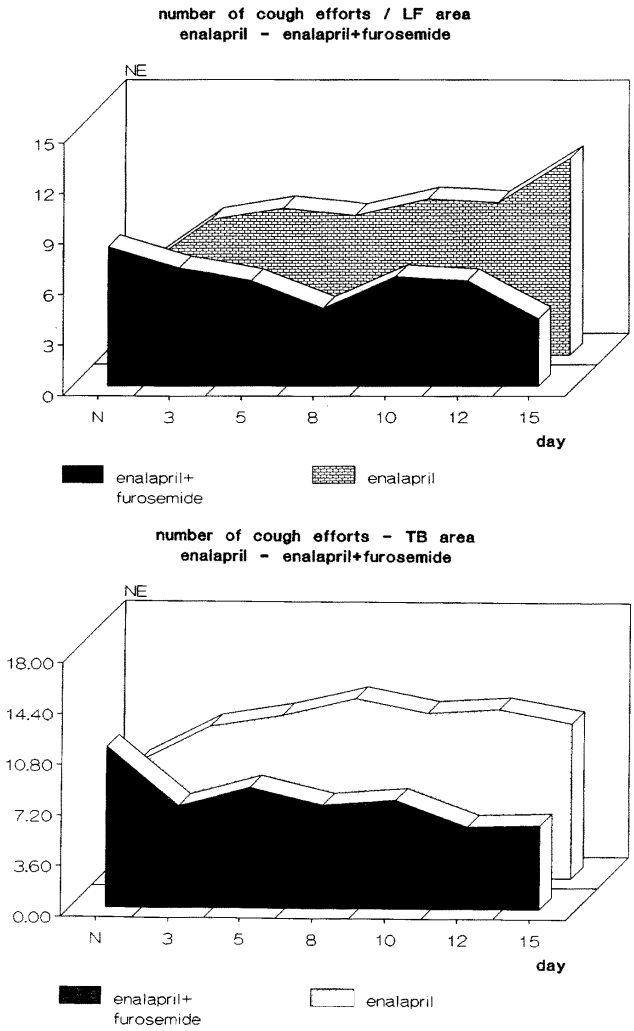


Fig. 3. The comparison of the number of cough efforts from laryngopharyngeal (LF) and tracheobronchial (TB) area of the airways, before drug administration (N), after 15-days enalapril administration and after 15-days administration of enalapril with inhaled furosemide.

smooth muscle to bronchoconstrictor mediators. The cat tracheal and lung smooth muscle reactivity, to the cumulative concentration of histamine (10^{-8}—10^{-4} mol.l^{-1}), was investigated after 15 days enalapril administration by the in vitro method. In our experiments the 15 days enalapril application resulted in the increase of amplitude of tracheal smooth muscle contraction to cumulative histamine doses, in comparison to control values. Opposite to the effect of the histamine on the tracheal smooth muscle, the reactivity of the lung smooth muscle was not affected significantly (Fig. 4).

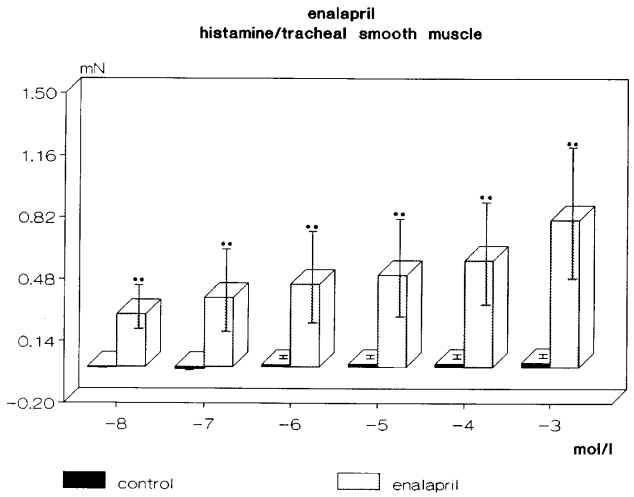
Simultaneous administration of enalapril with inhaled furosemide induced a significant decrease in the reactivity of the tracheal smooth muscle to bronchoconstrictor mediator — histamine. Changes in the reactivity of the lung smooth to the histamine were not observed after 15-days combined therapy (Fig. 5).

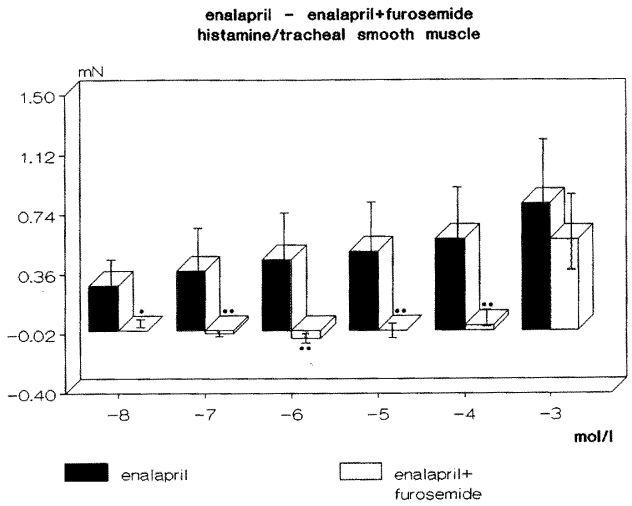
Discussion

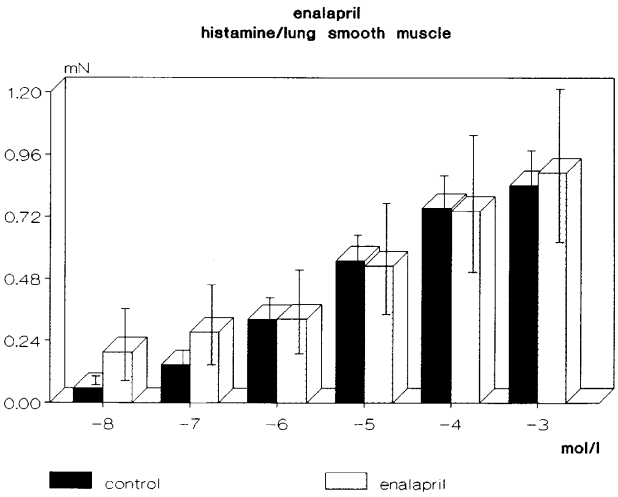
In our experimental conditions the 15 day application of enalapril in the dose of 5 mg/kg/day induced statistically significant increase in the parameters of a mechanically provoked cough, from laryngopharyngeal and tracheobronchial area of the airways. This negative influence of the respiratory tract, evoked by enalapril administration, was suppressed after the application of enalapril with inhaled furosemide.

Taking into consideration the basic goal of ACE-inhibitors applied and in order to struggle for elimination of their adverse effect to the respiratory system, our attention has been aimed at the inhaled furosemide. Furosemide is a loop diuretic, which alters ionic flux in other epithelial cells and in other neuronal tissue by inhibiting the $Na^+2Cl^-K^+$ co-transport (Bianco et al., 1993). Furosemide has a beneficial effect in modulation of the defence reflexes of the airways, and one of them is the inhibitory effect on the cough response induced by the wide range of the stimuli. In experimental conditions furosemide inhibited the cough induced by inhalation of low-chloride-content solutions in guinea pigs (Fox et al., 1995). It has been suggested that furosemide may be acting indirectly, perhaps by changing the concentration of Cl^--ions in pericilliary liquid and in this way is capable of altering the sensitivity of the cough receptors — RARs of the myelinated fibres and the endings of the unmyelinated C-fibres (Korpáš and Nosáľová, 1991). In addition, furosemide inhibits the cholinergic and noncholinergic-nonadrenergic transmission in the airways (Elwood et al., 1991).

In our experiments, focused on the comparison of the number of cough efforts during 15-day enalapril monotherapy and during 15 day simultaneous administration of enalapril with inhaled furosemide, we observed the significant decrease of the parameters of a mechanically induced cough after combined therapy enalapril







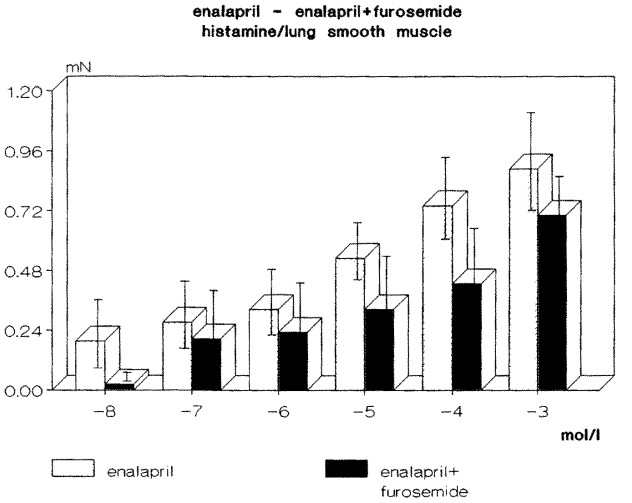


Fig. 4. The changes of the reactivity of the tracheal and lung smooth muscle to the cumulative doses of the histamine after 15 days enalapril administration. Black columns represent the normal values, the striped columns represent the values after 15-days administration of enalapril. The columns represent average values of the contraction amplitude with mean mistake of average±S.E.M. Axis x — the concentration of histamine in mol/l, axis y — the amplitude of the contraction in mN. Statistical significance was calculated according to Student's *t*-test ($^{*}p<0.05$, $^{**}p<0.01$).

Fig. 5. The changes of the reactivity of the tracheal and lung smooth muscle to the cumulative doses of the histamine after 15-days administration of enalapril with inhaled furosemide. Striped columns present the experimental values after enalapril administration. The checked columns represent the values after 15-days administration of enalapril with inhaled furosemide. The columns represent average values of the contraction amplitude with mean mistake of average±S.E.M.. Axis x — the concentration of histamine in mol/l, axis y — the amplitude of the contraction in mN. Statistical significance was calculated according to Student's *t*-test ($^{*}p<0.05$, $^{**}p<0.01$).

with furosemide. This finding showed that inhaled furosemide is able to suppress the enalapril induced cough. Because the mechanical irritation of the airways is a relatively selective stimulus for the induction of cough by the rapidly adapting receptors (Nosáľová, 1998), our results confirmed the participation of RARs either in the production of the cough after enalapril treatment or in the suppression of the cough reflex after furosemide inhalation.

Inhalation of furosemide has been shown to be effective in preventing bronchoconstriction induced by several stimuli such as exercise, allergens, bradykinin (Polosa et al., 1995), cold air, metabisulphate, adenosine (Bianco et al., 1993; Barnes, 1993) leukotriene LTD_4 (Cheung et al., 1990). The mechanism of this protective effect is unknown. Inhibition of bronchoconstriction could be related to inhibition of $Na^+2Cl^-K^+$ co-transport and Na^+/K^+ ATPase of the epithelial cells in the airways. The influencing the transport system probably does not play the primary role in bronchoprotective activity of furosemide. In patients with asthma, bumetanide, another loop diuretic with more potent inhibitory effects on the $Na^+2Cl^-K^+$ co-transporter, has no significant effect against either MBS or adenosine-induced bronchoconstriction (Vaghi et al., 1990).

Bronchoprotective effect of furosemide is blocked by non-steroidal antiinflammatory drugs (O'Connor et al., 1991). Therefore, one possible action of furosemide in the airways may be to stimulate the production of PGE_2 from the airway epithelium.

An additional beneficial factor in the mechanism of action of furosemide is the inhibition of mastocyte degranulation and histamine release in the airways (Finnerty et al., 1990). Because of the activation of mastocyte degranulation belong to secondary factors, which participate in pathomechanism respiratory adverse effects of ACE-inhibitors, this effect of furosemide could be beneficial in the suppression of the afore-mentioned complications. Inhibition of ACE cause the cumulation of bradykinin and substance P in the airways, which stimulate the mastocyte degranulation (Trifilieff et al., 1993). In clinical conditions Andersson and Persson (1994) and Bucknall et al. (1988) demonstrated the increase of bronchial reactivity to histamine in the patients treated with enalapril. These results correspond with our experimental findings, that 15 days enalapril administration caused the increase of amplitude of tracheal smooth muscle contraction to the histamine. It should be emphasised that the inhaled application of furosemide with enalapril resulted in significantly decrease tracheal smooth muscle reactivity to the histamine.

In conclusion, our experimental data provide consistent evidence of respiratory negative effects of ACE-inhibitors accompanied with increased incidence of mechanically induced cough and increased reactivity of the tracheal smooth muscle to the histamine. Simultaneous application of inhaled furosemide with enalapril showed that furosemide succeeded unambiguously in the restriction of the respiratory adverse effects of ACE-inhibitors.

Conclusions

1. 15-days administration of enalapril resulted in the significant increase of parameters of mechanically induced cough, from the laryngopharyngeal and tracheobronchial mucous membrane in non-anaesthetized cats.
2. Simultaneous application of enalapril with inhaled furosemide, in comparison to enalapril monotherapy, caused the decrease of the measured cough parameters.
3. After 15-days enalapril administration was registered the increase of amplitude of tracheal smooth muscle contraction to the histamine, by in vitro method.
4. The 15-days combined therapy (enalapril+furosemide) induced the significant decrease in reactivity of tracheal smooth muscle to histamine.
5. The reactivity of the lung smooth muscle to histamine was not affected significantly neither after 15-days enalapril monotherapy nor simultaneous application enalapril with inhaled furosemide.

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