TRACHEAL SMOOTH MUSCLE HYPERREACTIVITY INDUCED BY HYPOCHLORITE

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Summary

In vitro, several reactive oxygen species have been reported to induce constriction of the airways smooth muscle. The purpose of this paper was to study the reactive oxygen species (hypochlorite) effect on the tracheal smooth muscle in vitro and the prevention effect of N-acetyl-cysteine (NAC) and mepyramine (10⁻⁶ M), a histamine H₁-receptor antagonist. There were studied 12 spiral tracheas, obtained from male Wistar rats (200 g). A dose-response curve was performed using acetylcholine concentrations from 10⁻⁵ to 10⁻⁴ M. After 5 doses response curves and a washing period, samples were incubated for long term incubation (30 minutes) with hypochlorite (10⁻³ M) and a new acetylcholine dose-response curve was performed. Experiments were repeated after a preincubation with NAC 10⁻³ M for 20 min. The hypochlorite enhanced the acetylcholine constrictor effect (38 ± 2.9%) for all doses of acetylcholine (p<0.001). The preincubation with NAC prevented the constrictor effect induced by the hypochlorite (74±3.7%) (p<0.001). Hypochlorite induced a dose-response-dependent contraction of the tracheal smooth muscle. Addition of hypochlorite to the tracheal preparation induced a high, rapid contraction with a slow relaxation. NAC reduced the amplitude of contraction induced by hypochlorite (which is considered 100%) with 40± 2.4% (p<0.001) and an insignificantly effect was produced by mepyramine (a decrease of contraction induced by hypochlorite only with 2.8±1.03%) (p<0.05).

Key words: hypochlorite, acetylcholine, NAC, mepyramine, tracheal smooth muscle

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Introduction

Asthma is characterized by inflammation, airways constriction and hyperreactivity produced by the oxygen free radicals and inflammatory mediators during earlier and late phase asthmatic response (Chung et al., 2002; Doelman, 1991).

The first phase is characterized by bronchoconstriction and the second phase is determinate by a hyperresponsiveness to different stimuli as a result of an inflammatory process (Bogart et al., 2009; Tulic et al., 2002; Zosky, 2008) This inflammatory process can be modulated by the activation of immune reactions. These mechanisms were evaluated in clinical asthma but also in experimental model (Gunst et al., 2006; Mc Kinley et al., 2004; Nemzek et al., 2009).

The eosinophils play an important role in pathogenesis of late response and development of airways hyperresponsiveness. The cells release products like histamine, oxygen radicals; arachidonic acid metabolites are responsible for inflammatory process, bronchoconstriction and morphological changes of airways epithelium (Elias, 2000; Fredberg, 2000).

Eosinophils, mast cells and neutrophils are potent producers of reactive oxygen species like hydrogen peroxide and hypochlorite. Acid hypoclorous is an eosinophil peroxidase – H₂O₂-chloride product which determinates the contraction
of airways smooth muscle in a direct pathway and indirect by stimulation of the histamine release from mast cells and basophiles. Histamine can be also chlorinated at the amine position by the eosinophil peroxidase – \( \text{H}_2\text{O}_2\)-chloride system and product hypoclorous acid. These chloramines are agonists for histamine H1-receptors which are responsible for airways constriction. For this aspect, the selective histamine H1-receptor antagonists are used like therapeutic agent in asthma (Patil, 2011).

The purpose of this paper was to study the reactive oxygen species (hypochlorite) effect on the tracheal smooth muscle in vitro and the prevention effect of N-acetyl-cysteine (NAC) and mepyramine (10-6 M), a histamine H1-receptor antagonist (Kay, 2001).

Material and methods

There were studied 12 spiral tracheas, obtained from male Wistar rats (200 g). Preparations were put in an organ bath containing 50 ml Krebs-Hensellet solution at 37 Celsius degree, continuously gassed with a mixture of 95% O2 and 5% CO2 in order to maintain oxygen tension and a pH of 7.4.

A force transducer for recording isometric contraction, displayed on a xy inscriptor, has been used in order to follow up tracheal smooth muscle contractility. After an equilibrium period of 60 min with 6 intermediate changes of solution, a dose-response curve was performed using acetylcholine concentrations from 10-5 to 10-4 M. After 5 doses response curves and a washing period, samples were incubated for long term incubation (30 minutes) with hypochlorite (10-3 M) and a new acetylcholine dose-response curve was performed. Experiments were repeated after a preincubation with NAC 10-3 M for 20 min.

The tracheal preparations were washed for 30 min with six intermediate changes of buffer solution followed by an incubation procedure, 30 min with 10-3 M of hypoclurous acid in order to evaluate the influence of hypochlorite on the tracheal smooth muscle. The inhibition of hypochlorite constrictor effect was produced by NAC, respectively mepyramine (10-6 M), a histamine H1-receptor antagonist (before the incubation with hypoclurous acid).

Statistical analysis. For each study we have performed 4 to 6 experiments. Statistical analysis included calculation of mean values, standard deviation and Student’s test. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using Excel Microsoft Office 2007.

Results

The hypochlorite enhanced the acetylcholine constrictor effect (38 ±2.9%) for all doses of acetylcholine (p<0.001) (fig 1).

The preincubation with NAC prevented the constrictor effect induced by the hypochlorite (74±3.7%) (p<0.001) (fig 2).
Hypochlorite induced a dose-response-dependent contraction of the tracheal smooth muscle. Addition of hypochlorite to the tracheal preparation induced a high, rapid contraction with a slow relaxation after approx 30 minutes (fig 3).

Fig 3. The hypochlorite constrictor effect on rat tracheal smooth muscle

NAC reduced the amplitude of contraction induced by hypochlorite (which is considered 100%) with 40± 2.4% (p<0.001) and an insignificantly effect was produced by mepyramine (a decrease of contraction induced by hypochlorite only with 2.8±1.03%) (p<0.05) (fig 4).

Fig 4. The NAC prevention effect on tracheal smooth muscle constriction

**Discussion**

We evaluated the role of the reactive oxygen species (hypochlorite) in constriction of airway smooth muscle and hyperreactivity to muscarinic response, mechanism which appears in the late phase of the asthmatic response. Inhibition of hypochlorite action by N-acetyl-cysteine is useful in treatment of asthmatic late phase response characterized by airways hyperreactivity.

Hypochlorite can be formed by the action of eosinophils peroxidase, hydrogen peroxide and chloride ions.

The hypochlorite and the eosinophil toxic granule proteins are responsible for the epithelial damage, hyperresponsiveness and constriction of airways smooth muscle. Secondary, reactive oxygen species degranulates mast cells in order to release histamine and serotonin which are constrictor agents (Reddel et al., 2011; Solway, 2004).

The hypochlorite induced hyperreactivity of the rat tracheal smooth muscle to acetylcholine probably by the epithelial damage.

NAC reacts with hypochlorite and prevents the constriction of airway smooth muscle, effect which is not produced by the mepyramine - H1 histamine antagonist receptor (Que et al., 2005)

Results signalled out a relationship between doses and the incubation time for hypochlorite. The constrictor effect of hypochlorite is inhibited by NAC and has no effect the histamine H1-receptor antagonist.

Hypochlorite destructs the epithelial layer and this contributes to the genesis of the airway hypersensitivity to different stimuli and the constriction of airways smooth muscle.

Long term incubation for 30 min significantly enhanced the acetylcholine constrictor effect and is inhibited by NAC - a scavenger of reactive oxygen species.

**Conclusion**

Hypochlorite induces a hypersensitivity to acetylcholine which is due to destruction of epithelial layer. Hypochlorite modified the balance between muscarinic and β-adrenoceptor responses in rat tracheal strip in favour of the muscarinic response (functional antagonism).

**References**
