LONG-TERM ADMINISTRATION OF ORAI-1 ANTAGONIST HAS ANTITUSSIVE, BRONCHODILATORY AND ANTI-INFLAMMATORY EFFECTS IN EXPERIMENTAL ASTHMA

M. Sutovska[1], M. Kocmalova[1], M. Adamkov[2], M. Antosova, D. Vybohova[3], P. Mikolka[4], J. Hatok[5], S. Franova[1]

[1]Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Sklabinska street 26, 03601, Martin, kocmalova@jfmed.uniba.sk; sutovska@jfmed.uniba.sk; [2]Institute of Histology and Embryology, [3]Institute of Anatomy, [4]Institute of Physiology, and [5]Institute of Medical Biochemistry, Jessenius Faculty of Medicine, Comenius University, Mala Hora 4, 03601, Martin, Slovakia

Introduction: Intracellular calcium ($[Ca^{2+}]_i$) is mediating many essential functions including gene expression, chemotaxis, neurotransmitter release and contraction. Cellular $[Ca^{2+}]_i$ signals generally arise from the opening of $[Ca^{2+}]_i$ permeable ion channels, a diverse family of membrane proteins. One of them, store-operated channels (SOCs), are found in the plasma membranes of virtually all mammalian cells and are activated through a decrease in $[Ca^{2+}]_i$ in the endoplasmic reticulum. The best-studied SOC is a sub-type known as the Ca^{2+} release activated Ca^{2+} (CRAC) channel. CRAC channels are widely expressed in immune cells and generate Ca^{2+} signals important for gene expression, proliferation, and the secretion of inflammatory mediators. Contraction of airway smooth muscle (ASM), a key response underlying bronchoconstriction and cough in asthmatic airways, is dependent on both release from intracellular stores and influx through non–voltage-dependent pathways, e.g. receptor-operated channels (ROC) and SOC. Our previous study demonstrated the role of CRAC channels in contraction of ASM as well in pathophysiology of experimentally induced allergic asthma in guinea pigs. The aim of presented work was to evaluate the influence of long-term therapy by CRAC antagonist on airways hyperreactivity, pathological cough and degree of inflammation in experimental animal asthma model. **Material and methods:** Allergic inflammation of the airways was performed during 21 days and was induced by repetitive exposure of guinea pigs to allergen – ovalbumine, followed by 14 days lasted therapy by an antagonist of CRAC channels (3-fluoropyridine-4-carboxylic acid, Orai 1 inhibitor), administered intraperitoneally in the dose 1.5 mg/kg. The relaxing effect of CRAC antagonist was expressed as changes of specific airways resistance (sRaw) *in vivo* and contractile response of isolated ASM strips on contractile mediators, *in vitro* and was compared to salbutamol. The influence of CRAC antagonist and positive control drugs codeine on experimentally-induced cough reflex was presented as changes of coughs number. Citric acid aerosol (c=0.3 M) was used to provoke cough reflex in conscious guinea pigs. The assignment of exhaled NO levels (E_{NO}), the evaluation of NO-synthase isophorms levels by Real Time PCR method and immunohistochemical staining of tracheal and pulmonary tissue sections were used to verify an anti-inflammatory effect of Orai-1 inhibitor. **Results:** Long term application of CRAC antagonist resulted in significant cough suppression exceeded effect of control drug codeine, bronchodilatory effect *in vivo* and inhibited ASM contractility *in vitro* conditions higher than classic bronchodilatory drug salbutamol. Moreover, 14 days lasted therapy by CRAC antagonist decreased levels of E_{NO} nearly to values before sensitization and together with the results of immunohistochemical analysis validated anti-inflammatory effect of CRAC antagonist. **Conclusion:** Presented data confirmed antitussive, bronchodilatory and anti-inflammatory effect of long term application of CRAC antagonist. These finding supported the significant role of CRAC channels localized on ASM and immune cells in pathophysiology and symptoms of experimental asthma model. Therefore, CRAC channels represent promising target of new drugs for treatment of respiratory diseases causally associated with allergic inflammation of the airways.

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