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Imetit Dihydrobromide and Thioperamide Medication in Cough Hypersensitivity Model—The Role of H_3 Receptors

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Abstract

Chronic cough is a troublesome problem and it is frequently associated with diseases such as gastroesophageal reflux, asthma and upper airway diseases—so called diagnostic triade. The magnitude and severity of cough is strongly associated with the ongoing nasal inflammation in subjects with rhinosinusitis and treatment of nasal inflammation leads to the down regulation of pathologically up-regulated cough. Histamine plays a key role in the inflammation of the upper airways of different aetiologies; therefore histamine receptors seem to be promising targets. The aim of our study was to ascertain the effect of H_3R agonist imetit and H_3R antagonist thioperamide on cough and symptoms of allergic rhinitis (AR) in an animal model of upper airway cough syndrome in ovalbumin sensitized guinea pigs. OVA sensitized guinea pigs (n = 10) were repeatedly challenged with i.n. allergen-OVA to induce allergic rhinitis and to enhance cough reflex according to the validated model of experimental allergic rhinitis. Animals were pre-treated by i.p. administration of imetit (1 mg/kg and 2 mg/kg of body weight) and thioperamide 30 min. prior i.n. OVA administration. Rhinitis evaluation was based on the occurrence of typical symptoms. The effect on cough was assessed from the response to inhalation of citric acid (0.4 M, 10 min), final cough count and cough latency were analysed from the airflow traces, cough motor pattern and the cough sound. AR up-regulated the cough response from 9 ± 2 to 16 ± 1 cough per provocation, med ± IQR, $p < 0.05$ and shortened cough latency. Imetit (1 mg/kg) suppressed nasal symptoms and decreased number of cough from 16 ± 1 to 12 ± 1; however the data did not reach significance. Imetit (2 mg/kg) significantly suppressed the nasal symptoms, and number of coughs from 16 ± 1 to 6 ± 2,

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med ± IQR, $p < 0.05$. Thioperamide (5 mg/kg of body weight) did not have expected effects on tested parameters. H_3R agonist imetit, unlike H_3R antagonist thioperamide has antitussive potential and ability to suppress nasal symptoms in animal model of allergic rhinitis.

Keywords

Chronic Cough, Allergic Rhinitis, Histamine, Antitussive, Imetit, Thioperamide, H_3 Receptor

1. Introduction

Chronic cough (cough of more than 8 weeks duration) is a quite common presentation to both primary and secondary care, with prevalence in the community in a range of 9% - 33% [1]. The management of patients with chronic cough is often problematic, with relatively few therapeutic options. Many suffer long-term illness with a marked adverse effect on quality of life and major medical and socio-economic consequences [2]. Chronic cough has been associated with complex aetiology, but it is now considered to be rather "one syndrome" mainly mediated by hypersensitivity of cough-related airway afferents [3].

Upper airway disease (rhinosinusitis) is one of the most commonly identified causes of chronic cough. Animal models and studies in subjects with upper airway diseases showed that cough sensitivity correlates with the presence of nasal inflammation and magnitude of nasal symptoms. Effective treatments of local inflammation reduced significantly the magnitude of nasal symptoms and also reduced coughing and cough hypersensitivity [2] [4].

Nasal afferents play important role in modulation of cough reflex and histamine is a mediator that directly stimulates a subset of nasal sensory nerves [5]. The up-regulation of the neuronal mechanisms is clearly recognised as being a major component of the underlying aetiology of cough hypersensitivity. Local environment around airway afferents and/or the phenotype of these nerves may change in the airways of patients with chronic hypersensitivity syndrome. How this occurs, in which population of afferents and at which neuronal target is unclear. A currently favoured explanation is that inflammatory mediators could "sensitise" C-fibre afferent nerve endings (peripheral or central) leading to an increase in the electrical excitability of afferent nerves, thus reducing the threshold for activation [2]. Although intranasal administration of histamine did not trigger cough, it sensitised cough reflex in subjects with allergic rhinitis [6], the same affect was documented in healthy subjects [7].

Empiric protocols recommend first-generation H_1 antihistamines (inverse agonists of histamine H_1 receptor) for the treatment of UACS (upper airway cough syndrome), although mechanisms of their antitussive effect are not completely understood [8]. The use of antihistamines for cough so far lacked relevant evidence, but recently Dicpinigaitis and co-authors [9] clearly showed that old generation antihistamine diphenhydramine reduces cough reflex sensitivity in humans with cold. Unfortunately, old generation antihistamines cause adverse effects in multiple body systems, including sedation, caused by low selectivity for the H_1R and by the ability to cross the blood-brain barrier influencing H_1R in the CNS (central nervous system) [10]. In contrast to first-generation, second-generation H_1-antihistamines are relatively free from adverse effects however; they are ineffective in the treatment of cough in UACS [8].

Since the effect of histamine molecule is mediated not only by H_1R, but also by other receptor subtypes (H_2, H_3, H_4), we can assume that these subtypes of receptors can play a role in pathogenesis of nasal inflammation and up-regulation of cough reflex in subjects with nasal inflammation via neuronal mechanism.

In our study, we focused on histamine H_3 receptor (H_3R). H_3R acts as presynaptic neuronal autoreceptor (inhibits the release of histamine from histaminergic neurons) and heteroreceptor expressed on non-histaminergic neurons of central and peripheral nervous system, where it inhibits release of other neurotransmitters, such as acetylcholine, noradrenaline, dopamine and serotonin [11].

Expression of H_3R is expressed within nasal mucosa, not only on peripheral nerves [12], but also on epithelial cells, fibroblasts and around submucosal glands [13]. Studies indicate that this receptor plays an important role in pathophysiology of allergic rhinitis, in nasal obstruction, mucus secretion and inflammation [13]-[15].

Expression of H_3R significantly increases in infection by respiratory syncytial virus (RSV), which can be process responsible for airway hyperreactivity [13].

Stimulation of H_3R, which is expressed on nerve endings of sympathetic nervous system, inhibits release of noradrenaline from these endings. In absence of histamine, noradrenaline released from sympathetic nerve endings helps to maintain vascular tone. Presence of histamine decreases concentration of noradrenaline, which leads to vasodilation followed by nasal obstruction [16].

Furthermore, it was discovered that H_3R in lower airways in humans regulates cholinergic nerve transduction. H_3Rs are located on vagus nerve, where they modulate cholinergic neurotransmission. In airways they inhibit release of acetylcholine and can play role in modulation of neuronal bronchoconstriction in allergic diseases, when histamine is released in airway from mast cells in close proximity of ganglia and cholinergic nerves [17]. Histamine by its bond to H_3R on nerves containing neuropeptides influences function of mast cells.

H_3 receptors are subject of interest of preclinical and clinical drug discovery intended for airway allergy treatment. The question, whether in treatment of airway allergy diseases would be more effective H_3R agonists or antagonists remains unanswered.

Some studies suggest therapeutic applications of H_3R antagonists in treatment of allergic rhinitis. H_3 receptor antagonists showed ability to supress nasal symptoms of AR, especially nasal congestion in combination with antihistamines that target the H_1 receptor [18]-[23].

On the other hand, different studies showed, that H_3R agonists alleviated symptoms of allergic rhinitis and pathologic changes of nasal mucosa, probably due to inhibition of substance P release from sensory nerve endings [24] [25].

In our study we ascertain the effect of H_3R agonist imetit and H_3R antagonist thioperamide on symptoms of AR and on pathologically enhanced cough in an animal model of upper airway cough syndrome.

2. Methods

Study was conducted on male Dunking Hartley guinea pigs (n = 10), obtained from an accredited breeding facility (L. Sobota, Městec Králové, Czech Republic). The animals were housed in an approved animal holding facility maintained at a controlled room temperature of 21˚C - 22˚C, with humidity 60% - 70%, ventilation, a 12-h light-dark cycle and had free access to water and standard animal food.

Animal care was provided and the experiments were conducted in agreement with the Animal Welfare Guidelines of the Comenius University and statutes and rules of Slovak Republic legislation (protocol no. 3467/13 - 221).

Animals were adapted twice to laboratory conditions to reduce future stress. They stayed in a plethysmographic box to familiarize themselves with the environment and the laboratory staff. In the box, they were exposed to an aerosol of buffered saline for 2 minutes, which corresponds to the experimental procedure.

2.1. Sensitization

Animals were sensitised by intraperitoneal administration of 10 μg of OVA suspension together with aluminium hydroxide as an adjuvans in 1 ml of saline, OVA sensitivity was confirmed by skin prick tests 21 days later.

2.2. Assessment of Nasal Symptoms after Antigen Challenge

To obtain a relevant model of UACS, sensitised animals were repeatedly intranasally challenged by an allergen to induce allergic rhinitis thus to up-regulate cough reflex.

The symptoms intensity was evaluated by trained observers using a nasal symptom score system which was developed and validated by Brozmanova *et al*. [26] matching symptoms intensity to numeric values (maximum 6 and minimum 0).

Nasal symptoms score was calculated based on validated scale system. Observed signs and symptoms were count of sneezes and 1) nasal discharge: no discharge 0, mild/moderate 1, discharge falls in drops from nose 2; 2) eye/conjunctival reaction: no changes 0, hazy eyes 1, visible lacrimation 2; 3) nasal phenomenon: no crackles 0, audible crackles 1, crackles audible from a distance 2. OVA group received 0.5% OVA, 0.15 ml into both nostrils five times, once a week.

2.3. Citric Acid-Induced Cough in Alert Guinea Pigs

The awake animals were individually placed in the plethysmograph (type 855, Hugo Sachs Electronic, Ger-

many), which consisted of a head chamber and a body chamber. The opening between the head chamber and body chamber was equipped with a plastic collar lining around the animal's neck to prevent communication between the chambers. The appropriate collar size was chosen for each animal to prevent neck compression. The head chamber was connected to a nebulizer (Pari Provokation Test I, Menzel, Germany, manufacturer's specification: output 5 $l \cdot min^{-1}$, particle mass median aerodynamic diameter 1.2 μm). A suction device adjusted to the same input (5 $l \cdot min^{-1}$) was connected to the head chamber to maintain constant airflow through the chamber during the aerosol administration. Airflow changes were measured using a pneumotachograph (*Godart, Germany*) with a Fleisch head connected to the head chamber. The data were recorded with the acquisition system ACQ Knowledge (*Biopack, Santa Barbara, CA, USA*). Respiratory sounds, including sounds during coughing and sneezing, were recorded with a microphone placed in the roof of the head chamber and connected to a pre-amplifier and MP3 recorder. The pneumotachograph and microphone output were simultaneously recorded for off-line analysis.

The cough challenge was performed using an inhalation of 0.4 M citric acid for 10 min. Cough was defined as expiratory airflow interrupting the basic respiratory pattern accompanied by a coughing sound. Coughs were analysed (using cough-related sounds and airflow) by two trained persons who were blind to the treatment if any. Their results were compared and (if no statistically significant differences occurred) averaged.

The animals were challenged by citric acid (CA) and cough response was detected several times, always with a one week interval between the challenges to prevent tachyphylaxis. First measurement of cough response was performed after intranasal administration of saline solution (0.015 ml per nostril) 10 minutes prior cough challenge (negative control). In other measurements cough response was detected after intranasal administration of ovalbumin (0.5% sol. 0.015 ml per nostril) and induction of symptoms of allergic rhinitis (AR). Cough response was detected 10 min after intranasal administration of ovalbumin without pre-treatment twice (positive control), then after intraperitoneal pre-treatment with imetitin two different doses (1 mg/kg and 2 mg/kg of body weight), than after intraperitoneal pre-treatment with thioperamide (5 mg/kg of body weight) 30 min prior induction of rhinitis, what is 40 min prior cough challenge. In the end, reproducibility of experiments was repeated by measuring cough response after i.n. administration of 0.5% OVA without pre-treatment.

2.4. Reagents

Citric acid was purchased from Fisher (Slovak Republic). Ovalbumin and $Al(OH)_3$ were purchased from Fisher (Slovak Republic). Imetit and thioperamide were purchased from Tocris (Slovak Republic).

2.5. Statistical Analysis

For cough studies data non-parametric not-paired tests and multiple comparison tests were used as appropriate. Data for final count of coughs are expressed as median ± interquartile range; data for cough latency are shown as means ± SE. For statistical analysis, one-way ANOVA was used. A $p < 0.05$ was considered statistically significant.

3. Results

Presence of early phase of allergic rhinitis led to significant increase of cough per provocation (**Figure 1**) from 9 ± 2 to 16 ± 1, med ± IQR, $p < 0.05$ and significantly shortened cough latency from 189 ± 20 s to 66 ± 18 s (**Figure 2**). Pre-treatment by H_3R agonist imetit in dose of 1 mg/kg of body weight led to mild decrease of cough response and symptoms of allergic rhinitis. Imetit in dose of 2 mg/kg of body weight significantly suppressed symptoms of allergic rhinitis, decreased number of cough bursts from 16 ± 1 to 12 ± 1, med ± IQR, $p < 0.05$, and prolonged cough latency from 66 ± 18s to 192 ± 20, $p < 0.05$. Pre-treatment by H_3R antagonist thioperamide in dose of 5 mg/kg of body weight did not significantly influence symptoms of AR, number of cough bursts or cough latency (**Figure 1**, **Figure 2**).

4. Discussion

In our study, we have examined antitussive potential of H_3R agonist imetit and H_3R antagonist thioperamide. We found that imetit in dose 2 mg/kg of body weight in OVA-sensitized guinea pigs significantly decreased symptoms of AR and simultaneously significantly decreased number of citric acid induced coughs, whereas

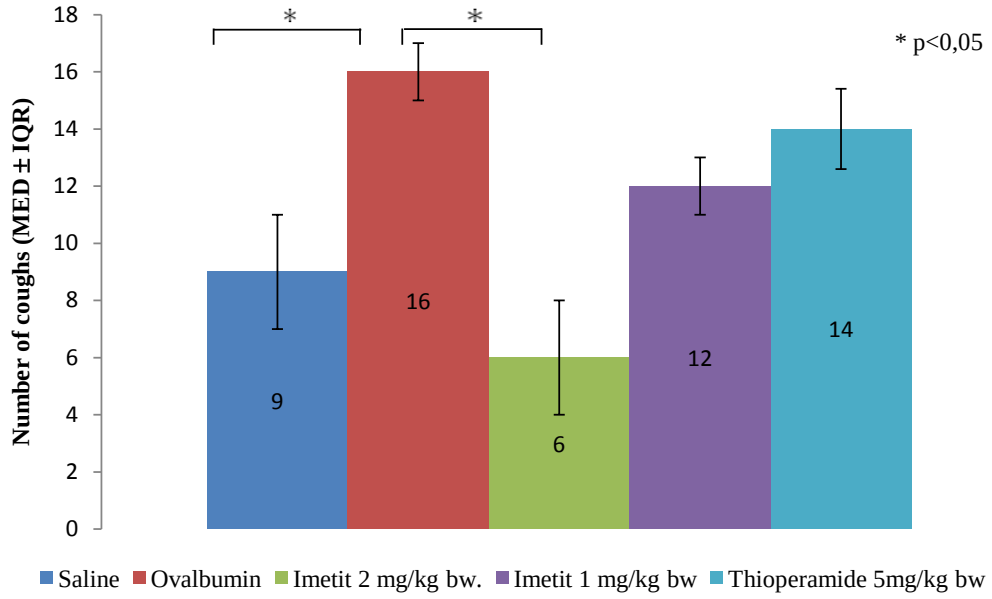

Figure 1. Effect of imetit and thioperamide on number of induced coughs.

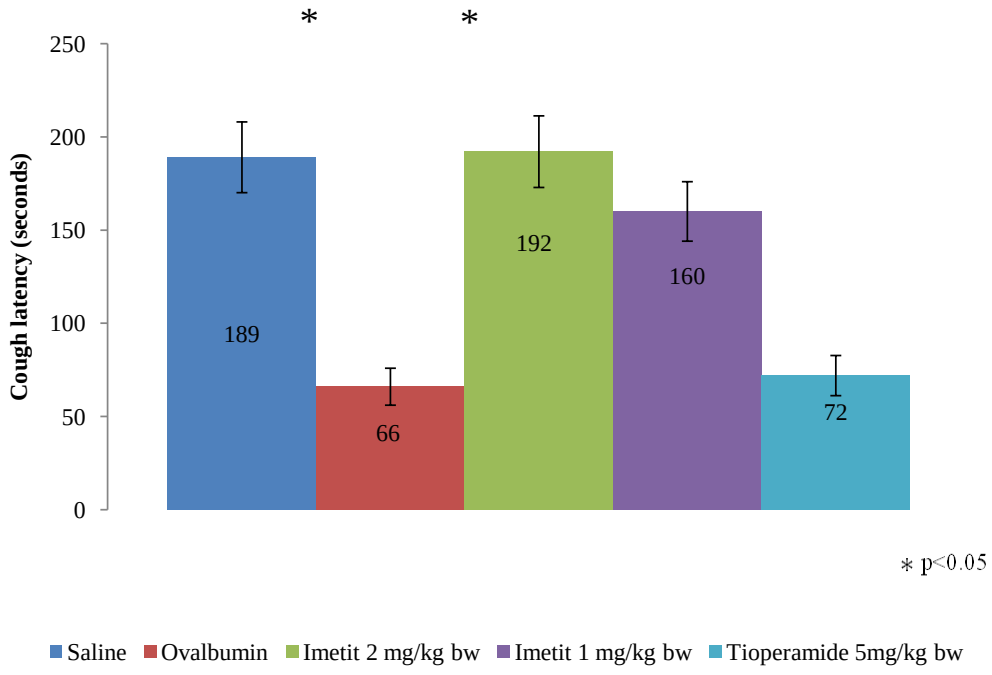

Figure 2. Effect of imetit and thioperamide on cough latency.

thioperamide proved to be ineffective in decreasing symptoms of AR and number of induced cough bursts.

As previously mentioned, H_3R is vastly expressed within central and peripheral nervous system, where it regulates release of histamine and many other important neurotransmitters. In allergic diseases of airways, histamine is released from mast cells in close proximity of ganglia and cholinergic nerves. H_3R inhibits release of acetylcholine, which can play role in process of neural bronchoconstriction [17]. Histamine by its bond to H_3R on nerves containing neuropeptides influences functions of mast cells. This local neuron-mast cell negative feedback loop plays role in process of neurogenic inflammation and its dysregulation can lead to excessive inflammation response [14]. Physiologic roles of H_3R in the airways, as its role in pathogenesis is not elucidated

enough. The fact, whether H_3R agonists or antagonists are of greater therapeutic potential, is dubious–studies in this area show quite controversial results.

On one hand, there are studies, which observed anti-inflammatory effect of H_3R agonists. In study conducted by Yang *et al*. [25] H_3R agonist imetit decreased symptoms of AR, pathologic changes of nasal mucosa and decreased concentration of substance P (SP) and its receptor (SP-R) in guinea pigs with experimentally induced allergic rhinitis. Similarly, Yokota *et al*. [24] in murine model of allergic rhinitis found out, that selective H_3R agonists—imetit and SCH 50971—significantly decreased nasal symptoms of allergy, possibly due to inhibition of SP release from sensory nerve endings. To similar conclusion led another study conducted by Sun *et al*. [27], in which imetit independently, as well as in combination with new-generation H_1R antagonist loratadine, successfully supressed symptoms of AR in guinea pigs, possibly due to decrease of SP secretion and expression of SP mRNA. In treatment of upper airway allergic diseases, H_3R agonists in combination with H_1R antagonists can be useful.

In our study, we have shown that imetit, aside of influencing nasal symptoms of AR, can supress hypersensitive cough, which often accompanies inflammatory diseases of upper airways. Antitussive effect of imetit, as well as other H_3R agonists, could be other convenient property of these substances and could broaden their clinical application.

Supposed mechanism, which could explain not only anti-inflammatory, but antitussive effect of imetit could be its interaction with SP. SP is one of pro-inflammatory neuromediators (tachykinins), which are locally released from sensory nerve endings in the nose (antidromic conduction) and contributes to pathogenesis of neurogenic inflammation and symptoms of rhinitis [28]. Release of neuropeptides can be induced by allergen, as well as histamine, prostaglandins and leukotrienes. Aside nerve endings; SP is produced by inflammatory cells (macrophages, eosinophils, lymphocytes, dendritic cells). It promotes proliferation and immunoglobulin production by lymphocytes, secretion of cytokines by lymphocytes, monocytes, macrophages and mast cells. By support of release of inflammatory mediators, such as cytokines, oxygen radicals, derivates of arachidonic acid and histamine, SP potentiates tissue damage, further stimulates leukocyte recruitment and thus increasing inflammatory response. Other effects of SP include vasodilation, increasing vascular permeability, and chemotaxis support, adhesion of leukocytes to endothelium—which leads to extravasation, migration and accumulation of leukocytes at site of inflammation. It also has mitogenic effects on smooth muscle cells, fibroblasts and endothelial cells [29].

Fang *et al*. [30] observed three times higher SP concentration in patients with AR when compared to healthy individuals. Similarly, Heppt *et al.* [31] observed increased expression of SP in nerve endings in patients with seasonal AR. Very interesting findings have been brought by a study conducted by Cho *et al*. [31]. In nasal lavage fluid of patients with chronic, non-productive cough and increased cough reflex sensitivity to inhalation challenge with capsaicin increased concentration of SP was observed. In these individuals, it can be case of C-fibre up-regulation, which could contribute to increased response to stimuli in other part of airways [28]. Similarly, Lim *et al*. [32] found out that patients with post-nasal drip syndrome accompanied by chronic cough have significantly increased concentration of SP in nasal secretion in comparison with patients with post-nasal drip without chronic cough. Influence of imetit on SP and its receptor could explain not only the ability to supress allergic rhinitis process, but the ability to desensitize hypersensitive cough in UACS, which was observed in our experiment. Contribution to antitussive effect of imetit can be mediated by not only on peripheral on level of nasal mucosa, but central as well-imetit penetrates blood-brain barrier into CNS, where H_3R is vastly expressed [33].

On the other hand, other studies suggest therapeutic applications of H_3R antagonists in treatment of allergic rhinitis. H_3 receptor antagonists showed ability to inhibit nasal congestion in combination with antihistamines that target the H_1 receptor [18]. Combined therapy by SCH 79687 (H_3R antagonist) and loratadine (H_1R antagonist) showed anti-allergic and decongestant properties [19]. In other studies, combined therapy by PF-03654746 (H_3R antagonist) and fexofenadine (H_1R antagonist) in patients with AR led to suppression of all nasal symptoms induced by allergen [20] [21]. Other H_3R antagonist, JNJ-39220675, administered per orally in dose 10 mg/kg of body weight in approximately 50 volunteers with AR improved overall score of nasal symptoms [22]. Similar results were obtained by Barchuk *et al*. [23]. Prophylactic treatment by JNJ-39220675 alleviated allergen-induced nasal congestion.

Histamine concentration in nasal cavity increases during inflammation processes in upper airways. Histamine inhibits release of noradrenaline via H_3R from sympathetic nerve fibres, which leads to vasodilation followed by

nasal obstruction [16] [34]. Suppression of this process by H_3R antagonists would explain their decongestant effects.

In our experiment, H_3R antagonist thioperamide in dose 5 mg/kg bw. i.p. was not effective in suppression of nasal symptoms of allergic rhinitis and did not exhibit ability to decrease number of citric acid-induced coughs in OVA-sensitized guinea pigs with induced AR. Similar results were obtained in a study conducted by Bolser *et al*. [35], where the effects of thioperamide (dose 3 - 10 mg/kg bw. s.c.) were tested on cough induced by capsaicin inhalation in healthy guinea pigs and inhalation of allergen in sensitized guinea pigs. Thioperamide did not supress cough neither in model of allergen-induced, nor capsaicin-induced cough. Neumann *et al*. [36] compared effect of thioperamide and H_4R antagonist JNJ 7777120 in murine model of ovalbumin-induced asthma. They found out that thioperamide does not have all useful properties of JNJ 7777120 in model of asthma. Thioperamide suppressed eosinophilia in bronchoalveolar lavage fluid, but in contrast to JNJ 7777120 did not decrease neither serum titre of specific IgE against OVA, nor inflammation infiltration of lung parenchyma.

5. Conclusion

In our study, H_3R agonist imetit, unlike H_3R antagonist thioperamide, dose-dependently suppressed nasal symptoms of AR, decreased number of cough bursts and prolonged cough latency. Based on our experiments we assume that H_3R agonists have higher therapeutic potential than H_3R antagonists in treatment of cough in UACS.

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Conflict of Interest

Authors do not declare conflict of interest.

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