



Review article

The potential of methylxanthine-based therapies in pediatric respiratory tract diseases

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ABSTRACT

Caffeine, theophylline and theobromine are the most known methylxanthines as they are present in coffee, tea and/or chocolate. In the last decades, a huge experimental effort has been devoted to get insight into the variety of actions that these compounds exert in humans. From such knowledge it is known that methylxanthines have a great potential in prevention, therapy and/or management of a variety of diseases. The benefits of methylxanthine-based therapies in the apnea of prematurity and their translational potential in pediatric affections of the respiratory tract are here presented.

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Abbreviations: AOP, Apnea of prematurity; IBMX, 3-isobutyl-1-methylxanthine; PDEs, phosphodiesterases; cAMP, cyclic AMP; CNS, central nervous system; CYP1A2, 1A2 isoenzyme of the cytochrome P450; FDA, Food and Drug Administration; IgE, Immunoglobulin E; NAEPP, National Asthma Education and Prevention Program; EPR3, Expert Panel Report 3; WHO, World Health Organization; IL-10, Interleukin 10; ECRHS, European Community Respiratory Health Survey; SARs, Slowly Adapting Stretch Receptors; COPD, Chronic Obstructive Pulmonary Disease.

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1. Introduction

Diseases of the upper or lower respiratory tracts include a group of severe and disabling pathologies that affect a high percentage of the World population. Air pollution and the increase of environmental allergens have contributed in the last decades to a significant increase in respiratory-related morbidity and mortality. The incomplete development of the respiratory system and the immaturity of respiratory control are major issues in prematures and, accordingly, the majority of emergency room visits and hospitalizations in children are related to respiratory problems. Apart from classical treatments, which include a wide range of prescription drugs, natural compounds such as the methylxanthines in coffee and cacao have protective effects on the respiratory system. The present article focuses on the potential of methylxanthines in diseases of the airways with increasing prevalence in children, in particular in asthma, cough and the apnea of prematurity (AOP).

2. Methylxanthines in natural sources

Methylxanthines are natural components of cacao-related products or non-alcoholic beverages as coffee, tea or yerba mate. Methylxanthines are also present in cola drinks and in several plant species as *Camellia pitilophylla* (cocoa tea), *Paullinia cupana* (guaraná) or *Citrus* sp., used to prepare dietary supplements [1,2]. Seven natural methylxanthines have been so far identified: aminophylline, 3-isobutyl-1-methylxanthine (IBMX), paraxanthine, pentoxifylline, theobromine, theophylline and caffeine. The latter three have been more extensively studied due to their presence in coffee, tea and/or chocolate.

Caffeine concentration in coffee cups, which depends on the coffee plant varieties and the preparation method, ranges from 57 mg/100 ml in a cup made from grounded and toasted coffee to 2 mg/100 ml in decaffeinated preparations [3]. Coffee also has theobromine and theophylline but at a 20-fold lower concentration [4]. Preparations from yerba mate leaves (*Ilex paraguariensis*), known as mate tea, contain the highest concentration of theophylline followed by black and green tea [4,5]. Finally, theobromine is the most abundant methylxanthine in cacao (*Theobroma cacao*) and cacao by-products, where the theobromine amount is directly related with the percentage of cacao, e.g. in dark chocolate more than in milk chocolate [6].

3. Physiological effects of methylxanthines

3.1. Mechanism of action

Four different cellular mechanisms of action have been described for methylxanthines: mobilization of intracellular calcium, inhibition of phosphodiesterases (PDEs), modulation of GABA_A receptors and antagonism of adenosine receptors (Fig. 1). Recent evidence however points to further modes of action that may be relevant in translational research.

Methylxanthines affect the concentration of the two main

second messengers in mammals, cyclic AMP (cAMP) and Ca²⁺. On the one hand, methylxanthines facilitate the release of Ca²⁺ from the endoplasmic reticulum in both skeletal and cardiac muscle. On the other hand, methylxanthines increase cAMP levels through the inhibition of PDEs [7]. Due to structural similarity with purine nucleosides, caffeine, theophylline and theobromine can act as competitive inhibitors of adenosine receptors. In humans there are four widely distributed sub-types of these G-protein coupled receptors, A₁, A_{2A}, A_{2B} and A₃. In fact, every cell in the human body has at least one subtype of adenosine receptors. Cells in many tissues express one or several of these four subtypes. For instance, smooth muscle and endothelial cells may express A₁, A_{2A} and A_{2B} receptor subtypes, whereas skeletal muscle mainly expresses A_{2A} and A_{2B} receptor subtypes, A_{2A} in type 1 and A_{2B} in type 2 fibers [8]. The A₃ subtype is less studied but its expression has been reported in lungs and liver [9], brain [10], colon [11], eosinophils [12] and dendritic cells [13]. Adenosine receptor blockade seems to be the responsible for many of the central nervous system (CNS) effects of methylxanthines. Concentrations reached after dietary intake are high enough to block adenosine receptors.

3.2. Methylxanthine metabolism

Theobromine and theophylline are intermediate molecules of caffeine metabolism that has been studied in animal models but, more predominantly, in man. Following oral administration, gastrointestinal absorption of caffeine is fast and complete, reaching almost 100% of bioavailability. Once it reaches the bloodstream, where it binds to albumin, caffeine is distributed in all tissues by simple diffusion or carrier-mediated transport. Caffeine metabolism is hepatic and mainly mediated by the 1A2 isoenzyme of the cytochrome P450 (CYP1A2), which is the responsible for the between-subject variability of caffeine plasma concentration [14]. The half-life of caffeine is 4.1 h, but it may be higher during pregnancy, in patients with hepatic diseases or in infants and neonates (up to 100 h) [15]. The main products of the first steps in caffeine metabolism are paraxanthine, theobromine and theophylline (Fig. 2) [16]. Due to its actions on CYP1A2 expression, some lifestyles –as smoking– increase caffeine clearance [17]. Theobromine, theophylline and the final product, methyluric acid, are excreted via urine. Although theobromine is toxic for a variety of mammals, the incidence of toxicity in humans is virtually absent; toxic events may occur when methylxanthines are taken in combination with dietary supplements for weight loss [18,19] or with drugs of abuse [20].

4. Methylxanthines for AOP therapy

AOP is the most common problem in premature neonates; it refers to a period of time longer than 20 s with no breathing, often accompanied by hypoxia, bradycardia, cyanosis, pallor and/or severe hypotonia. It usually affects infants born at a gestational age of 37 weeks or less [21]. The incidence of apnea is inversely related to gestational stage [22]: 7, 15, 54 and 100% at gestational ages of, respectively, 34–35, 32–33, 30–31 and less than 29 weeks. As the

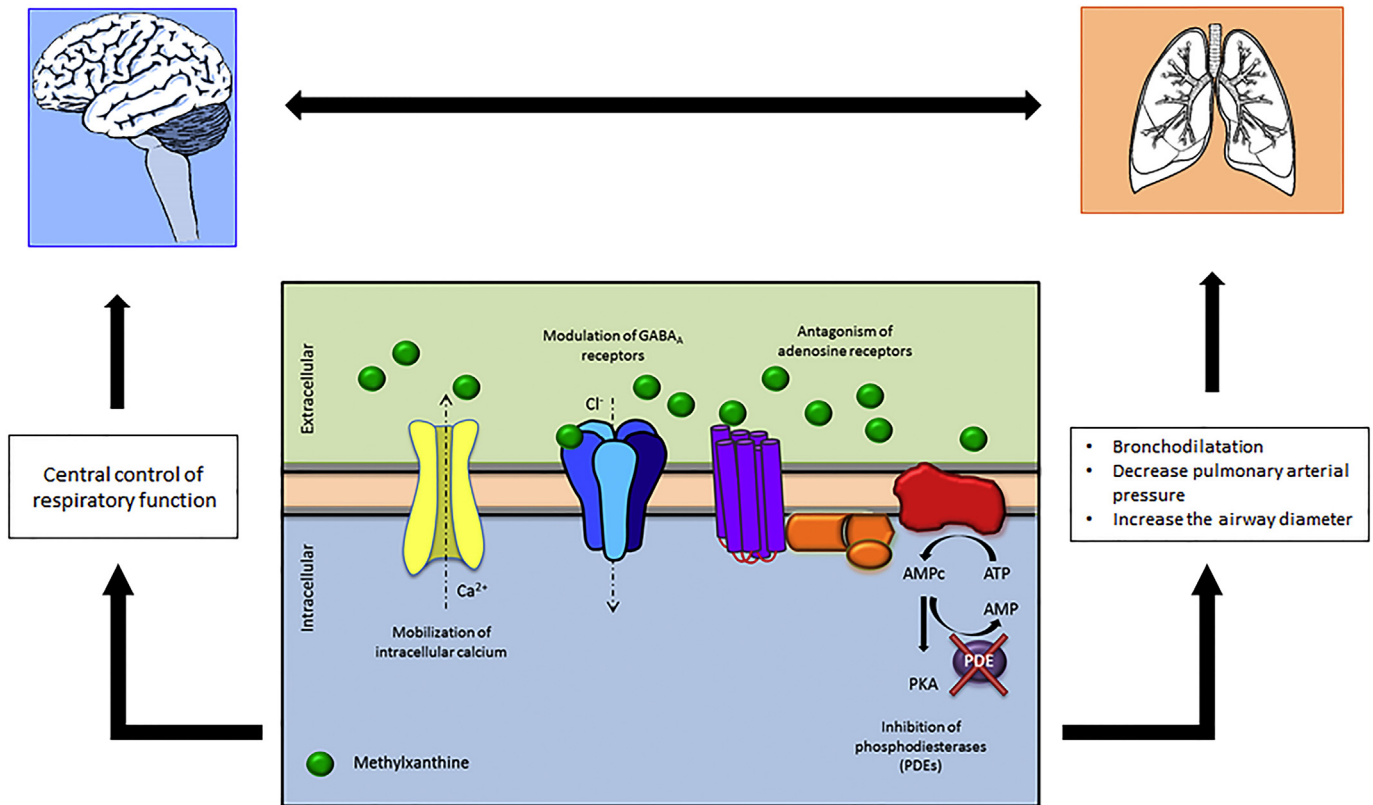


Fig. 1. Molecular mechanisms and physiological actions of methylxanthines.

infant's lungs and central respiratory control mature, AOP resolves such that it is more of a developmental disorder rather than a disease [23].

AOP is traditionally classified as central, obstructive or mixed. Central apnea is characterized by cycles of disrupted breathing during sleep, obstructive apnea exhibits regular respiratory thoracic movements but inadequate nasal air flow, and the mixed type results from a combination of both [24]. Central apnea accounts for approximately 40% of all cases of apnea, obstructive apnea accounts for a 10% and the mixed type accounts for a 50% [25].

Several theories regarding the pathogenesis of AOP have been reported but none is fully accepted. Hypercapnia detected by central chemoreceptors may be resumed by the increase of ventilation and by the increase in tidal volume and breathing frequency [26]. Premature infants may not be able to afford these automatic responses due, for instance, to failure in proper respiratory muscle control. In fact, some *in vivo* studies have demonstrated a differential threshold for hypercapnia in preterm infants and that contraction of diaphragm before that of upper airway muscles favors AOP [27,28]. It should be noted that the difference between basal pCO₂ and pCO₂ triggering apneic responses is very small in neonates. In this sense, minor oscillations in breathing in preterm infants may lead to abnormal respiratory homeostasis [29,30]. The hyperactive laryngeal chemoreflex, in response to pharyngeal reflux of gastric content, constitutes another possible cause for infantile apnea [31,32].

4.1. Caffeine and theophylline in the therapy of AOP

The clinical management of AOP includes prevention, supportive measures and pharmacological and oxygen therapies.

Supportive measures imply prone position, tactile stimulation, continuous positive airway pressure, and/or mechanical ventilation, whereas prevention comes from an appropriate posture of the neck and the maintenance of body temperature between 36.5 and 37 °C [33–35]. One of the tried interventions, blood transfusion to increase oxygen transport capacity, has been shown ineffective [35–37]. In contrast, both theophylline administration and CO₂ inhalation are effective [34,38]. A classical therapy to reduce the amount of apneas consists of inhalation of CO₂-containing air for 2 h. Despite clinical benefits in reducing the apneic time and rate from 9 s/min and 94 episodes/hour to 3 s/min and 34 episodes/hour, inhalation of CO₂-containing air suffers one limitation related with the quickly accommodation to the CO₂ concentration in inspired air [35,37,38].

Among all the pharmacological treatments, methylxanthines such as caffeine, theophylline and aminophylline, are a good choice to treat AOP [33,34]. Kuzenko (1973) and Kuzenko and Paala (1973) reported the benefits of rectal administration of aminophylline in the therapy of AOP [39,40]. These findings were confirmed later by Bednarek and Roloff [41] and Shannon et al. [42] that introduced the therapeutic use of oral administration of theophylline. Several studies have been undertaken to improve the effectiveness of this treatment. The optimal therapeutic doses of theophylline are 4 mg/kg and 1.5 mg/kg in, respectively, neonates and preterm neonates. Optimal plasma concentration should be in the 8–10 µg/ml range, but plasma levels higher than 14–16 µg/ml lead to undesirable side effects like sleeplessness, hyperglycemia, hypertension and/or heart arrhythmias [43].

Methylxanthine therapy is a mainstay of treatment of central apnea by stimulating the CNS and respiratory muscle function [44–46]. As a non-selective antagonist of adenosine receptors, caffeine may improve minute ventilation, CO₂ sensitivity,

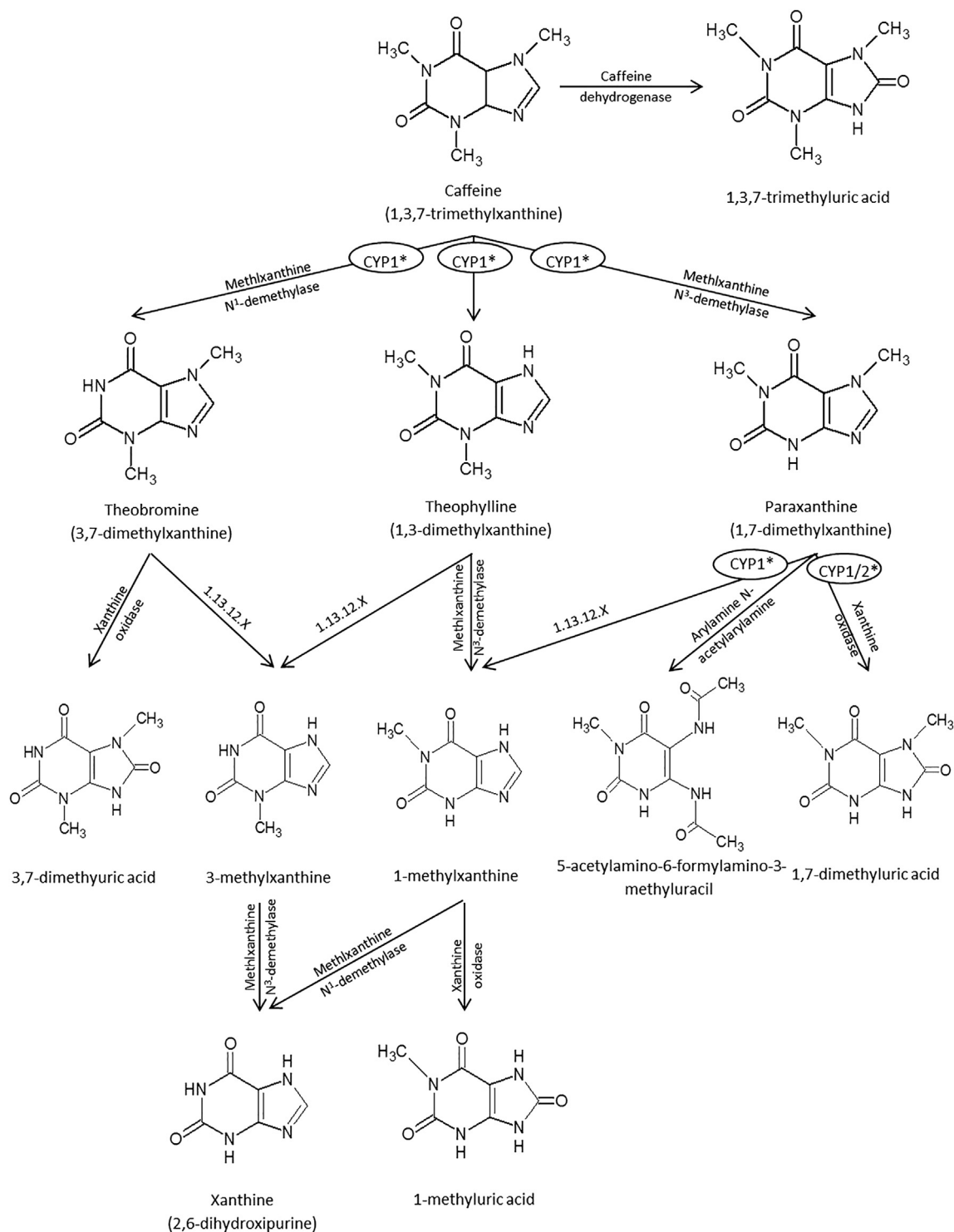


Fig. 2. Scheme of caffeine metabolism in man. The name of the enzyme catalyzing each reaction is provided on each arrow. A not yet identified enzyme, 1.13.12.X, belongs to the family of oxidoreductases acting on single donors and using a single oxygen atom (IUPAC, Enzyme Commission). * CYP Cytochrome p450; family 1, 2 and/or 3.

contraction of the diaphragmatic muscles and neural respiratory regulation [33,47]. These benefits of caffeine in decreasing the frequency of apneic episodes in infants were first demonstrated in late 70 s [48]. However, it was not until 2000, when the Food and Drug Administration (FDA) approved caffeine for the treatment of AOP, that this methylxanthine emerged as a good alternative to theophylline. Caffeine citrate can be administrated orally or

intramuscularly with a recommended initial dose of 20 mg/kg, followed by a daily maintenance dose of 5 mg/kg [49,50]. It is well known that methylxanthines cross the blood brain barrier and, accordingly, central actions impact on CO₂ sensitivity of chemoreceptors, diaphragm and respiratory muscle contraction and on catecholamine-induced responses [25]. As mentioned above, all these anti-AOP actions may be due to blockade of adenosine

receptors, especially of A₁ and A_{2A} [51].

Caffeine, theophylline, and aminophylline are non-selective antagonists of adenosine receptors, being adenosine a centrally-acting respiratory depressant involved in the inhibition of respiratory drive [52–54]. In this sense, some authors demonstrated, in newborn piglets, that methylxanthines acting on adenosine A_{2A} receptors expressed in GABAergic neurons can prevent the inhibition of laryngeal chemoreflex induced by GABA release [40,55]. The possible role of adenosine A₁ receptors in this phenomenon cannot be ruled out.

Epidemiological evidence shows both theophylline and caffeine as highly successful molecules at reducing apneic episodes of neonates by acting on the CNS [56]. A comparative study demonstrated that caffeine performs better than theophylline in terms of efficacy, peripheral side effects or drug clearance (Table 1). In fact, theophylline is generally less well tolerated and more prone to cause tachycardia and/or gastrointestinal dysfunction. In contrast, caffeine has a faster onset of action, lesser fluctuations in plasma concentration, longer half-life and fewer peripheral side effects [23,57].

5. Methylxanthines for asthma therapy

Asthma is a chronic respiratory disease that usually starts at a young age. It is characterized by inflammation and constriction of the airways with episodes that can be life-threatening. The main symptoms are wheezing and serious coughing periods that severely affect the ability to breathe [58]. Despite asthma was described 2000 years ago, anti-inflammatory drugs were not used until the 1960 s [59].

An expert panel commissioned in 2007 by the National Asthma Education and Prevention Program (NAEPP), developed a guideline for the diagnosis and management of asthma, namely the “Expert Panel Report 3” (EPR 3). According to the World Health Organization (WHO) estimation, there are 235 million people with asthma, mainly children. Despite the effective therapies, asthma is a deadly disease, especially in lower-middle income countries where this pathology is under-diagnosed and poorly managed due to the high cost of the available therapies [60].

Main risks are airway immaturity, genetic predisposition and the environmental exposure to substances and particles that may cause allergic reactions and/or irritation. Triggers may be quite diverse: medicines (i.e. aspirin), chemical irritants in the workplace (i.e. detergents or metals), household allergens (i.e. dust or pet dander), outdoor allergens (i.e. pollen and mold), extreme emotional arousal (i.e. anger or fear) and strenuous physical exercise [59]. Several explanations have been provided such as association of inflammation of airways in asthma with allergic sensitization. The respiratory tract is able to recognize common environmental allergens and generate a specific and exacerbated immunologic response that can induce an immunoglobulin E (IgE)-dependent inflammatory process mediated by histamine, tryptase, leukotrienes and/or prostaglandins [61,62]. Other factors

influencing the immune system maturation and impacting on the prevalence of allergic and autoimmune diseases in childhood are infectious agents [63].

5.1. Theophylline and doxofylline in the therapy of asthma

Management of asthma includes both, effective treatment of acute attacks and long-term control medications. Standard therapies to alleviate bronchoconstriction and asthma symptoms while decreasing the frequency and severity of asthma episodes include short- and long-acting β -adrenergic agonists (e.g. formoterol and salmeterol), anti-cholinergic agents and inhaled corticosteroids (e.g. salbutamol, terbutaline, formoterol, salmeterol, fluticasone, beclomethasone, budesonide, or ciclesonide) [64]. Other treatments with moderate benefits include leukotriene antagonists (e.g. montelukast) and long-acting muscarinic antagonists (e.g. tiotropium or aclidinium). Although currently available anti-asthma drugs exhibit good results in terms of efficacy, they show some limitations related with adherence, tolerability, adverse side-effects and/or high cost [65]. Inhaled corticosteroid therapy, for instance, is not effective in all asthmatic patients; some children do not respond to steroids whereas others show a substantial decrease of the beneficial effects within months after corticosteroids are discontinued [66–68]. Adherence to the therapies is also problematic due to undesirable effects, such as decrease in bone density, alterations in adrenal function or cataracts [69–72]. It is known that theobromine and caffeine improve lung function and produce bronchodilatation in asthma patients [73,74]. Also, it has been demonstrated that patients with asthma and bronchitis may self-administer coffee or chocolate due to perceived symptomatic relief [25].

Theophylline has been used for various decades in the treatment of asthma and is still among the most prescribed drugs; it is effective and relatively inexpensive [75]. Theophylline has a bronchodilator action and may decrease inflammation underlying asthma [76]. Moreover, theophylline also produces a relaxation in airways and of vascular smooth muscle thus decreasing the mean pulmonary arterial pressure [77]. These actions are particularly relevant when asthma is not sufficiently controlled by inhaled corticosteroids with or without concomitant long-acting β_2 -adrenergic agonists. In these situations theophylline is used as an effective add-on therapy [60].

In prospective randomized clinical trials, in cohorts of patients with severe asthma, low-dose addition of theophylline to inhaled corticosteroids results in a better disease management than doubling the dose of corticosteroids or than the use of β_2 -adrenergic agonists [75,78,79]. PDEs inhibition is the most accepted mechanism of action for theophylline in asthma pathology. Muscle contraction is regulated by intracellular levels of cAMP, which is synthesized by adenylate cyclases and hydrolyzed by PDEs. Rabe et al., in 1995 demonstrated that theophylline is able to relax the inherent tone of human bronchial rings at concentrations similar to those inhibiting cAMP hydrolysis by PDEs [77]. Specifically, theophylline induces bronchodilatation by inhibition of PDE3 activity whereas its anti-inflammatory effect may be due to inhibition of PDE4 together with histone deacetylase-2 activation [80]. Adenosine receptor antagonism [81] or increase of interleukin 10 (IL-10) [82] and the nuclear translocation of the nuclear factor kappa-light-chain-enhancer of activated B cells [83] are further proposed mechanisms. Beneficial effects only take place at high concentrations in therapies that use theophylline alone. In this sense, the conventional dosing strategy consisting of oral administration of 200–400 mg twice per day may cause adverse effects. An excessive inhibition of adenosine receptors could produce gastrointestinal (stomach ache, nausea or diarrhea), CNS

Table 1
Caffeine and theophylline in the treatment of apnea of prematurity.

Variable	Caffeine	Theophylline
Efficacy	+++	+++
Peripheral side effects	+/-	+++
Drug clearance (t _{1/2} , hours)	100	30
Plasma level at steady state	Stable	Fluctuating
Dosing interval	Once a day	1–3 times per day

Adapted from Ref. [56] (t_{1/2}: time required for reducing the plasma concentration of the drug to a half).

(headache, irritability or difficulties concentrating at work) or cardiovascular (irregular heart rate or palpitations) manifestations. A synthetic methylxanthine, doxofylline, which differs from theophylline by the presence of a dioxolone group in position 7, has been developed to limit undesirable side effects. Different studies in animal models as well as in humans have found that this drug shows similar efficacy as theophylline but with less adverse effects [84]. In this sense, the lower affinity to A₁ and A₂-adenosine receptors that shows doxofylline may be related to these less adverse reactions [85–89].

6. Methylxanthines in cough management

Cough is a protective reflex that helps in keeping airway integrity and prevents lung infections. However, a reduction in the threshold for reflex initiation leads to cough in response to stimuli that are normally innocuous. Persistent coughing is a health problem that impairs life quality and causes chest pain, loss of bladder control or headedness [90,91]. Furthermore, infections that affect the upper (common cold, flu, laryngitis or sinusitis) or the lower (bronchitis and pneumonia) tract often lead to episodes of severe coughing. Non-infectious causes of chronic cough include allergic rhinitis, asthma, hypertension or cardiovascular diseases and require pharmacological treatment of the underlying condition [92]. Despite of the idea that this reflex is a normal body reaction, the prevalence of chronic cough is high (18%). The European Community Respiratory Health Survey (ECRHS) estimated that the incidence is higher in females than in males and is linked to pathologies like asthma or obesity and/or to unhealthy lifestyles like tobacco smoking [93].

Along the respiratory tract, neurons expressing C-fibers and rapidly and slowly adapting stretch receptors (SARs) can be activated in response to mechanical and physico-chemical stimuli (acid, temperature), or natural irritants (capsaicin) [94,95]. The reflex impulse arrives to the cerebral cough center, located in the upper brain stem and pons, via the vagus nerve. Vagus, phrenic, and spinal motor nerves constitute the efferent pathway arriving to diaphragm, abdominal wall and expiratory muscles and generate a coordinated response [96].

6.1. Theobromine to alleviate persistent coughing

The pharmacological management of cough is complex. The inhibition of the cerebral cough center by codeine is one of the most effective treatments. This opiate may be used alone or in combination with anti-inflammatory or antipyretic drugs. Due to undesirable effects, codeine prescription is contraindicated in newborn infants and in children undertaking surgery; its use in adults is also decreasing. Apart from eliminating or, at least, reducing the exposition to irritants, expectorants and mucolytic drugs decrease the viscosity of bronchial secretion thus facilitating its release. Acetylcysteine, for instance, is able to break disulfide bonds in mucoproteins, whereas other drugs, ambroxol or carbocysteine, can modulate the viscoelastic properties of the mucus [97,98]. Menthol increases the excitability threshold in peripheral reflexogenic areas, distending peripheral blood vessels and alleviating bronchoconstriction [99]. Finally, anti-inflammatory drugs (non-steroidal anti-inflammatory drugs or cyclooxygenase inhibitors) decrease respiratory airway inflammation by inhibiting the production of chemical mediators such as prostaglandin or cyclooxygenase [100–102].

Growing evidence in the last years suggests that theobromine may be a novel and promising antitussive treatment for patients with either acute or chronic coughing. On the other hand, it can be used as a bronchodilator owing to the fact that the administration of methylxanthines increases the airway diameter [103].

Some *in vivo* and *in vitro* studies performed by Usmani et al., in 2005, suggest that theobromine is able to inhibit cough in a direct way, thus behaving as an antitussive drug. The authors observed that concentrations of theobromine showing no signs of adverse effects inhibit citric acid-induced cough in guinea-pigs. Interestingly, the methylxanthine was able to inhibit capsaicin-induced cough in a cohort of patients undertaken a randomized, double-blind, placebo controlled study. Moreover, experiments in isolated human and guinea-pig vagus nerve preparations demonstrated that theobromine directly inhibits sensory afferent nerve activation [104]. This is in accordance with the recently described ability of methylxanthines to regulate the activation of human intermediate-conductance Ca²⁺-activated potassium channels that are key in controlling afferent and efferent vagal activity [105,106]. Interestingly, it has been reported that cacao intake may reduce cough. Taken together this observation and the enhanced bioavailability of methylxanthines in natural sources, dark chocolate consumption appears as an attractive way to administer theobromine to patients with cough [107]. Despite theobromine use is a novel alternative to the controversial opioid treatment, more studies are needed to know the effects of daily intake of this methylxanthine, especially in children in whom an appropriate balance between toxicity risk and therapeutic benefit is critical [108].

7. Methylxanthine-based therapy in other respiratory diseases: COPD and bronchiectasis

In view of the demonstrated benefits of methylxanthines in serious respiratory tract pathologies such as AOP, asthma and cough, some laboratories are testing the translation potential of these natural compounds or synthetic analogs in other lung diseases, namely chronic obstructive pulmonary disease (COPD) and bronchiectasis.

COPD is a disorder caused by airway tract inflammation that may lead to serious difficulties in breathing. The WHO considers it the fifth cause of mortality in the World and predicts that could reach the second place in 2030 [109]. The principal causes of COPD are related with unhealthy habits as smoking and long-term exposures to polluted air, chemical vapors or dust. The heavy air pollution in some populated Chinese cities affects lung function and exacerbates COPD in both adults and children [110]. However, COPD is usually diagnosed in adult age. Interestingly, theophylline and the synthetic xanthine derivate doxofylline, have been recommended for the treatment of this disorder with positive results and similar proven efficacy [111].

Bronchiectasis is an uncommon and irreversible stretching and widening of the bronchial tree that can be congenital or secondary to other pathological processes as tuberculosis, tumors or bacterial (*Klebsiella pneumoniae*, *Staphylococcus aureus* or *Pseudomonas aeruginosa*) and viral (adenovirus) infections [112,113]. Currently, it has no cure and the therapeutic management focuses on relieving symptoms and improving the life quality of patients. The aims of the treatment depend on the initial event, e.g. a specific antibiotic will be prescribed if the cause is a bacterium. However, if the bronchial damage is extensive, oxygen therapy and hydration or respiratory exercises are suggested along with bronchodilators or corticosteroids. Based on the proven benefits in other respiratory diseases and on its anti-inflammatory properties, theophylline is gaining attention for bronchiectasis therapy. The results of clinical trials performed with methylxanthines are not yet conclusive and more placebo controlled clinical studies are needed [114]. To assess the effect of theophylline in bronchiectasis, one clinical trial whose primary outcome is the score of the St. George's respiratory questionnaire is currently recruiting patients [see Ref No NCT01684683

in: <https://clinicaltrials.gov/>].

8. Conclusions

AOP, asthma and cough are examples of common respiratory problems that affect preterm infants and children and whose incidence in the past decades has increased dramatically. Acute respiratory events in the early life are likely to be important in the development of chronic lung diseases that affect the physical health of the children, but also their social, emotional, and academic performance. Classical therapies including corticosteroid or opiate administration and inhalation of CO₂-containing air, display clinical benefits but suffer from limitations related with adherence, adverse side effects and/or high cost. In this context, methylxanthines arise as natural compounds capable of stimulate CNS and respiratory muscle function. Individually administered or as add-on therapy to other interventions, solid evidence proves the methylxanthine efficacy in a variety of respiratory pediatric diseases. The excellent bioavailability and the experience from using methylxanthine-containing nutrients may benefit a more extended use in children for which pleasant taste and medication adherence are correlated.

Conflicts of interests

The authors declare no conflict of interest.

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