Theophylline

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Theophylline (dimethylxanthine) has been used to treat airway diseases for more than 80 years. It was originally used as a bronchodilator, but the relatively high doses required are associated with frequent side effects, so its use declined as inhaled β_2-agonists became more widely used. More recently it has been shown to have antiinflammatory effects in asthma and chronic obstructive pulmonary disease (COPD) at lower concentrations. The molecular mechanism of bronchodilatation is inhibition of phosphodiesterase (PDE) 3, but the antiinflammatory effect may be due to inhibition of PDE4 and histone deacetylase-2 activation, resulting in switching off of activated inflammatory genes. Through this mechanism, theophylline also reverses corticosteroid resistance, and this may be of particular value in severe asthma and COPD, wherein histone deacetylase-2 activity is reduced. Theophylline is given systemically (orally as slow-release preparations for chronic treatment and intravenously for acute exacerbations of asthma). Efficacy is related to blood concentrations, which are determined mainly by hepatic metabolism, which may be increased or decreased in several diseases and by concomitant drug therapy. Theophylline is now usually used as an add-on therapy in patients with asthma not well controlled on inhaled corticosteroids with or without long-acting β_2-agonists and in patients with COPD with severe disease not controlled by bronchodilator therapy. Side effects are related to plasma concentrations and include nausea, vomiting, and headaches due to PDE inhibition and at higher concentrations to cardiac arrhythmias and seizures due to adenosine A_1-receptor antagonism. In the future, low-dose theophylline may be useful in reversing corticosteroid resistance in COPD and severe asthma.

Keywords: methylxanthine; phosphodiesterase; adenosine receptor; histone deacetylase

HISTORICAL INTRODUCTION

Theophylline is still one of the most widely prescribed drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD) worldwide, because it is inexpensive and widely available. Theophylline (dimethylxanthine) occurs naturally in tea and cocoa beans in trace amounts. It was first extracted from tea and synthesized chemically in 1895 and initially used as a diuretic. Its bronchodilator property was later identified, and it was introduced as a clinical treatment for asthma in 1922. Despite its widespread global use, in industrialized countries theophylline has become a third-line treatment as an add-on therapy in patients with poorly controlled disease, because inhaled β_2-agonists are far more effective as bronchodilators, and inhaled corticosteroids have a greater antiinflammatory effect. Theophylline is used as an oral therapy (rapid or slow-release tablets) or as more soluble aminophylline, an ethylenediamine salt, which is suitable for oral and intravenous use (1).

MECHANISMS OF ACTION

Several molecular mechanisms of action have been proposed for theophylline (Table 1), although many of these occur only at higher concentrations ($>10^{-5}$ M) than are clinically effective.

Phosphodiesterase Inhibition

Theophylline is a weak nonselective inhibitor of phosphodiesterase (PDE) isoenzymes, which break down cyclic nucleotides in the cell, leading to increased intracellular concentrations of cAMP and cyclic 3′,5′ guanosine monophosphate concentrations (Figure 1). However, the degree of inhibition is small at therapeutic concentrations. Theophylline relaxes airway smooth muscle by inhibition mainly of PDE3 activity, but relatively high concentrations are needed for maximal relaxation (2), and its inhibitory effect on mediator release from alveolar macrophages is mediated by inhibition of PDE4 activity (3). Inhibition of PDE should lead to synergistic interaction with β-agonists, but this has not been convincingly demonstrated *in vivo* or in clinical studies. Inhibition of PDEs accounts for the most frequent side effects of theophylline.

Adenosine Receptor Antagonism

Theophylline antagonizes adenosine A_1 and A_2 receptors at therapeutic concentrations but is less potent at A_3 receptors, suggesting that this could be the basis for its bronchodilator effects. Although adenosine has little effect on normal human airway smooth muscle *in vitro*, it constricts airways of patients with asthma via the release of histamine and leukotrienes, suggesting that adenosine releases mediators from mast cells of patients with asthma via A_{2B} receptors (4). Inhaled adenosine causes bronchoconstriction in subjects with asthma via release of histamine from airway mast cells, and this is prevented by therapeutic concentrations of theophylline, although this does not signify that this is important for its antiasthma effect. However, adenosine antagonism is likely to account for the serious side effects of theophylline, such as seizures and cardiac arrhythmias, via blockade of A_1 receptors.

Increased IL-10

IL-10 has a broad spectrum of antiinflammatory effects, and its secretion is reduced in asthma and COPD. IL-10 release is increased by relatively high concentrations of theophylline mediated though PDE inhibition (5), although this has not been seen at the low doses that are effective in asthma (6).

Effects on Transcription

Theophylline prevents the translocation of the proinflammatory transcription factor nuclear factor-κB (NF-κB) into the nucleus

(*Received in original form February 26, 2013; accepted in final form May 3, 2013*)

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Am J Respir Crit Care Med Vol 188, Iss. 8, pp 901–906, Oct 15, 2013

Originally Published in Press as DOI: 10.1164/rccm.201302-0388PP on May 14, 2013
Internet address: www.atsjournals.org

TABLE 1. PROPOSED MECHANISMS OF ACTION OF THEOPHYLLINE

Phosphodiesterase inhibition (nonselective)
Adenosine receptor antagonism (A_1, A_{2A}, A_{2B} receptors)
Inhibition of nuclear factor-κB (↓ nuclear translocation)
↑ Histone deacetylase 2 via Inhibition of phosphoinositide 3-kinase-δ
↑ IL-10 secretion
↑ Apoptosis of inflammatory cells (neutrophils, T cells)
↓ Poly(ADP-ribose)polymerase-1 (PARP-1)

through preventing the degradation of the inhibitory I-κBα, thus potentially reducing the expression of inflammatory genes in asthma and COPD (7). However, these effects are seen at high concentrations and are likely to be mediated by inhibition of PDE.

Effects on Cell Survival

Theophylline promotes apoptosis in neutrophils *in vitro* through a reduction in the antiapoptotic protein Bcl-2 (8). Theophylline also induces apoptosis of T lymphocytes, thus reducing their survival, and this effect appears to be mediated via PDE inhibition (9). Theophylline also inhibits the enzyme poly(ADP-ribose)polymerase-1 (PARP-1), which is activated by oxidative stress and leads to a reduction in NAD levels, resulting in an energy crisis that leads to cell death (10).

Histone Deacetylase Activation

Theophylline in low therapeutic concentrations (~5 mg/L) activates histone deacetylases, especially when their activity is reduced by oxidative stress (11, 12). In COPD cells, in which HDAC2 activity and expression are markedly reduced, theophylline (10^{-6} M) restores HDAC2 activity to normal and thus reverses the corticosteroid resistance in these cells, an effect that is blocked by an inhibitor of HDAC activity trichostatin A (12) (Figure 2). This action of theophylline is independent of PDE inhibition and adenosine receptor antagonism but due to selective inhibition of phosphoinositide-3-kinase-δ (PI3K-δ) that is activated by oxidative stress and involved in the inhibition of HDAC2 activity via phosphorylation (13). Increased reactive oxygen species and nitric oxide from increased expression of inducible nitric oxide synthase result in the formation of peroxynitrite radicals, which nitrate tyrosine residues in HDAC2, resulting in its inactivation and degradation (14). Theophylline reduces the formation of peroxynitrite, thus providing a further mechanism for increasing HDAC2 function in asthma and COPD (15).

PHARMACOKINETICS

There is a close relationship between the acute improvement in airway function and serum theophylline concentrations. Below 10 mg/L bronchodilator effects are small, and above 25 mg/L additional benefits are outweighed by side effects, so that the therapeutic range was usually taken as 10 to 20 mg/L (55–110 μM). Nonbronchodilator effects of theophylline may be seen at plasma concentrations of less than 10 mg/L, so it is preferable to redefine the therapeutic range as 5 to 15 mg/L. The dose of theophylline required to achieve therapeutic concentrations varies among patients, largely because of differences in clearance. For children (6–12 yr), one-half of the adult dose should be used. Theophylline is rapidly and completely absorbed, but there are large interindividual variations in clearance, due to differences in its hepatic metabolism (Table 2). Theophylline is metabolized in the liver by the cytochrome P450 microsomal enzyme system, and a large number of factors may influence hepatic metabolism. Theophylline is predominantly metabolized by CYP1A2, whereas at higher plasma concentrations CYP2E1 is also involved (16). Increased clearance is seen in children (1–16 yr) and in cigarette and marijuana smokers. Concurrent administration of phenytoin, phenobarbitone, or rifampicin, which increase P450 activity, increases metabolic breakdown, so that higher doses may be required. Reduced clearance is found in liver disease, pneumonia, and heart failure, and doses need to be reduced to one-half and plasma levels monitored carefully. Decreased clearance is also seen with several drugs, including erythromycin, quinolone antibiotics (ciprofloxacin, but not ofloxacin), allopurinol, cimetidine (but not ranitidine), serotonin uptake inhibitors (fluvoxamine), and the 5-lipoxygenase inhibitor zileuton, all of which interfere with CYP1A2 function. Thus, if a patient on maintenance theophylline requires a course of erythromycin, the dose of theophylline should be halved. Although there is a similar interaction with clarithromycin, there is no interaction with azithromycin (17). Viral infections and vaccinations (influenza immunizations) may also reduce clearance, and this may be particularly important in children. Because of these variations in clearance, individualization of theophylline dosage is required, and plasma concentrations should be measured 4 h after the last dose with slow-release preparations, when steady state has usually been achieved.

PHARMACODYNAMICS

Theophylline has several cellular effects that may contribute to its clinical efficacy in the treatment of asthma and COPD (Figure 3).

Bronchodilator Action

Theophylline was primarily used as a bronchodilator, and it relaxes large and small human airways *in vitro*, acting as a functional antagonist by increasing intracellular cAMP concentrations. However, it is a relatively weak bronchodilator at therapeutic concentrations, with little bronchodilator effect at plasma concentrations of less than 10 mg/L. *In vivo* intravenous aminophylline has an acute bronchodilator effect in patients with asthma, which is most likely to be due to a relaxant effect on airway smooth and has a small protective effect of theophylline on histamine-, methacholine-, or exercise-induced bronchospasm. Oral theophylline reduces air trapping in patients with COPD, indicating an effect on peripheral airways (18).

Antiinflammatory Effects

Theophylline has several antiinflammatory effects in asthma and COPD, and these may be seen at lower plasma concentrations

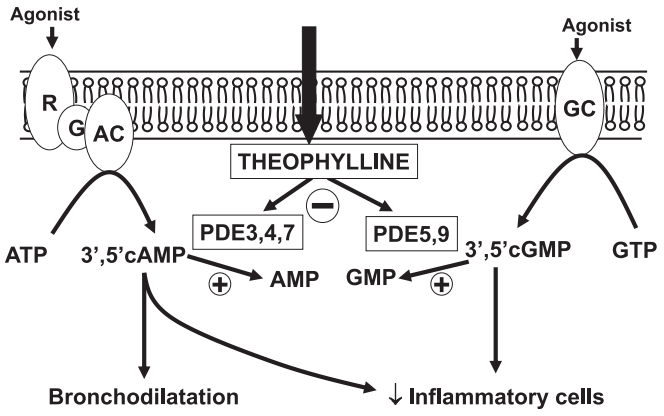

Figure 1. Effect of phosphodiesterase (PDE) inhibitors in the breakdown of cyclic nucleotides in airway smooth muscle and inflammatory cells. AC = adenylyl cyclase; cGMP = cyclic guanosine monophosphate; G = stimulatory G-protein; GC = guanylyl cyclase; GTP = guanosine triphosphate; R = receptor.

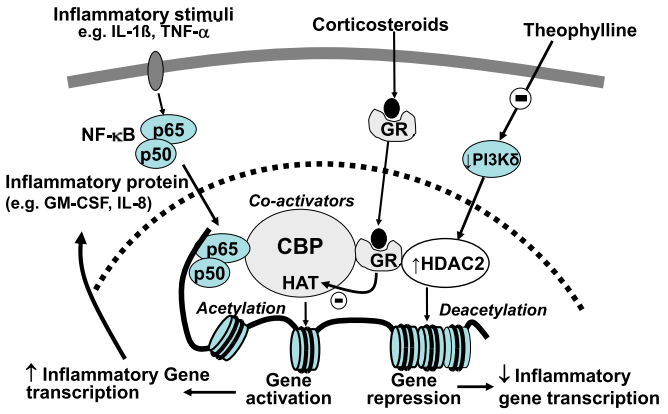

Figure 2. Theophylline increases histone deacetylase-2 (HDAC2) via inhibition of phosphoinositide-3-kinase-δ (PI3Kδ), which is activated by oxidative stress to phosphorylate and reduce HDAC2. HDAC2 deacetylates core histones that have been acetylated by the histone acetyltransferase (HAT) activity of coactivators, such as CREB-binding protein (CBP). This results in suppression of inflammatory genes and proteins, such as granulocyte-macrophage colony stimulating factor (GM-CSF) and CXCL8, that have been switched on by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB). Corticosteroids also activate HDAC2, but through a different mechanism, resulting in the recruitment of HDAC2 to the activated transcriptional complex via activation of glucocorticoid receptors (GR), which function as a molecular magnet. This explains how theophylline may reverse corticosteroid resistance due to reduced HDAC2 activity.

than are required for its bronchodilator actions (19). *In vitro* theophylline inhibits mediator release from mast cells and reactive oxygen species from neutrophils, although this is significant only at relatively high concentrations. Low-dose theophylline reduces the late response and airway eosinophil influx after inhaled allergen (20) and reduces the numbers of eosinophils in bronchial biopsies, bronchoalveolar lavage, and induced sputum in patients with mild asthma (21). It also reduces bronchoalveolar lavage neutrophil influx in patients with nocturnal asthma (22).

In patients with COPD, theophylline reduces the proportion of neutrophils in induced sputum and reduces the concentration of CXCL8, suggesting an antiinflammatory effect unlike corticosteroids (23–25). At high concentrations, theophylline inhibits proliferation in $CD4^+$ and $CD8^+$ lymphocytes and inhibits the chemotactic response of T lymphocytes, effects that are mediated through PDE inhibition (26). In patients with asthma, low-dose theophylline treatment results in an increase in activated circulating $CD4^+$ and $CD8^+$ T cells but a decrease in these cells in the airways, suggesting that it may reduce the trafficking of activated T cells into the airways (27).

Extrapulmonary Effects

Aminophylline increases diaphragmatic contractility and reverses diaphragm fatigue (28), but this effect has not been observed by all investigators. However, there are doubts about the relevance of these observations to the clinical benefit provided by theophylline (29). Whether theophylline has any effects on systemic effects or comorbidities in patients with COPD has not yet been established.

CLINICAL USE

Acute Exacerbations

Intravenous aminophylline was previously widely used in the management of acute exacerbations of asthma and COPD. However, in patients with acute asthma a systematic review showed no evidence of benefit when added to nebulized β_2-agonists for any outcome measure, whereas there was an increased risk of side effects (30), and similar results were found in children (31). Intravenous aminophylline should be reserved for the few patients with acute severe asthma who fail to show a satisfactory response to nebulized β_2-agonists. When intravenous aminophylline is used, it should be given as a slow intravenous infusion with careful monitoring of vital signs, and plasma theophylline concentrations should be measured before and after infusion. Aminophylline similarly has no place in the routine management of COPD exacerbations (32, 33).

Chronic Asthma

Currently, theophylline is recommended as an additional bronchodilator if asthma remains difficult to control after high doses of inhaled corticosteroids plus long-acting β_2-agonists (LABAs) (34). In an open study of adolescent patients with severe asthma controlled with oral and inhaled steroids, nebulized β_2-agonists, inhaled anticholinergics, and sodium cromoglycate, in addition to regular oral theophylline, withdrawal of the theophylline resulted in a marked deterioration of asthma control, which only responded to reintroduction of theophylline (35). In a placebo-controlled trial of theophylline withdrawal in patients with severe asthma controlled on high doses of inhaled corticosteroids, there was a significant deterioration in symptoms and lung function when placebo was substituted for the relatively low maintenance dose of theophylline (27). Addition of theophylline improves asthma control to a greater extent than β_2-agonists in patients with severe asthma treated with high-dose inhaled corticosteroids (36). Several studies have demonstrated that adding low-dose theophylline to inhaled corticosteroids in patients whose asthma is not controlled gives better asthma control than doubling the dose of inhaled corticosteroids (37–39). Interestingly, there is a greater degree of improvement in FVC than in FEV_1, suggesting an effect on air trapping and peripheral airways. The improvement in lung function is relatively slow, suggesting an antiinflammatory rather than a bronchodilator effect of theophylline. These studies suggest that low-dose theophylline may be preferable to increasing the dose of inhaled steroids when asthma is not controlled on moderate doses of inhaled steroids; such a therapeutic approach would be less expensive than adding a LABA, although it is less effective (34). Low-dose theophylline is also effective in smoking patients with asthma, who have a poor response to inhaled steroids, and this may be through

TABLE 2. FACTORS AFFECTING CLEARANCE OF THEOPHYLLINE

Increased clearance
P450 enzyme induction by drugs (rifampicin, phenobarbitone, carbamazepine, ethanol)
Smoking (tobacco, marijuana)
High-protein, low-carbohydrate diet
Barbecued meat
Childhood
Decreased clearance
P450 enzyme inhibition by drugs (cimetidine,* erythromycin,† fluoroquinolone antibiotics, allopurinol, zileuton, fluvoxamine, phenytoin, fluconazole, ketoconazole, acyclovir, ritonavir, diltiazem, verapamil, interferon-α, estrogens, pentoxifylline)
Congestive heart failure
Liver disease
Pneumonia
Viral infection
Vaccination (influenza immunization)
High carbohydrate diet
Old age

* Not ranitidine.
† Also clarithromycin but not azithromycin.

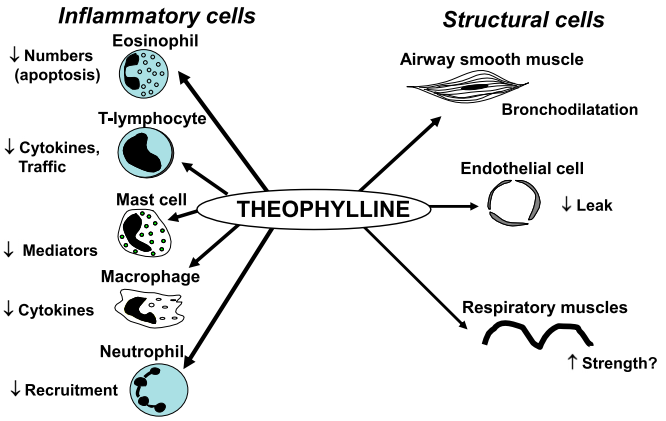

Figure 3. Cellular effects of theophylline.

increasing HDAC2 activity, which is reduced in the airways patients with asthma who smoke (40); this has been confirmed *in vitro* (41).

Chronic COPD

Theophylline increases exercise tolerance in patients with COPD (42) and reduces air trapping (18). In high doses (with plasma concentration 10–20 mg/L) it is a useful additional bronchodilator in patients with severe COPD and has an added effect to a LABA (43). Low-dose theophylline reduces exacerbations in patients with COPD by approximately 50% when used as single therapy over 1 year (44). In COPD macrophages, theophylline restores HDAC2 activity to normal and thus reverses corticosteroid resistance (12). Low-dose theophylline increases the recovery from acute exacerbations of COPD, and this is associated with reduced inflammation and increased HDAC activity (45). In patients with moderate COPD, low-dose theophylline has a greater antiinflammatory effect and improvement in FEV_1 when added to an inhaled corticosteroid than either drug alone (46). This suggests that theophylline may be useful in reversing corticosteroid resistance in patients with COPD, and long-term clinical trials are currently underway in patients with COPD to investigate this.

Apnea in Preterm Infants

Theophylline has been used to prevent recurrent apnea and bradycardia in preterm infants by stimulating breathing. It is effective in reducing episodes and the need for mechanical ventilation, although less effective than caffeine (47).

DOSING STRATEGIES

Intravenous

Intravenous aminophylline has long been used in the treatment of acute exacerbations of asthma and COPD but is used much less now as it is less effective than nebulized β_2-agonists. The recommended dose is 6 mg/kg given intravenously over 20 to 30 minutes, followed by a maintenance dose of 0.5 mg/kg/h. If the patient is already taking theophylline, or there are any factors that decrease clearance, these doses should be halved and the plasma level checked more frequently.

Oral

Plain theophylline tablets or elixir are rapidly absorbed but give wide fluctuations in plasma concentrations and are not recommended. Several sustained-release preparations of theophylline and aminophylline that are absorbed at a constant rate provide steady plasma concentrations over a 12- to 24-hour period (1). The recommended doses for bronchodilatation are 200 to 400 mg twice daily, but one-half of these doses may be effective as an anti-inflammatory treatment. Although there are differences between preparations, these are relatively minor. Once optimal doses have been established, plasma concentrations usually remain stable, providing no factors that alter clearance are introduced.

Other Routes

Aminophylline may be given as a suppository, but rectal absorption is unreliable and proctitis may occur, so this route should be avoided. Inhalation of theophylline is irritating and ineffective (48). Intramuscular injections of theophylline are very painful and should never be given.

COST-EFFECTIVENESS

Slow-release theophylline preparations are less expensive than LABA as an add-on therapy, although less effective. Plain theophylline is very inexpensive but not recommended because of fluctuation in plasma concentrations.

COMBINATION THERAPY

At present there are no fixed combination therapies available. In the future, a combination of low-dose theophylline and an oral corticosteroid might be useful in COPD.

MEASURING EFFECTS AND OUTCOMES

Assessment of the effect of adding theophylline to existing therapy usually involves demonstrating an increase in FEV_1, which occurs rapidly with a bronchodilator effect (43) but may occur more slowly due to an antiinflammatory effect (37). Typically, the increase in FEV_1 is accompanied by a decrease in symptoms. In COPD, theophylline may reduce air trapping and improve exercise performance, although its effects are small (49). The reduction in exacerbations reported in patients with COPD is difficult to monitor in clinical practice (44).

ADVERSE EFFECTS

The main limitation to the use of theophylline in conventional doses has been the relatively high frequency of adverse effects. Unwanted effects of theophylline are usually related to plasma concentration and tend to occur when plasma levels exceed 20 mg/L, although patients develop side effects at low plasma concentrations. Side effects may initially be reduced by gradually increasing the dose until therapeutic concentrations are achieved.

The most frequent side effects are headache, nausea and vomiting, increased acid secretion, and gastroesophageal reflux, which may be explained by PDE inhibition. Diuresis may be due to adenosine receptor antagonism. At high concentrations, convulsions and cardiac arrhythmias may occur and may be due to adenosine A_{1A}-receptor antagonism. Doxofylline, which is available in some countries, is another methylxanthine derivative that has similar efficacy to theophylline but appears to have less effect on adenosine receptors so may be safer (50).

SAFETY SYSTEMS

It is important to recognize the factors that both increase and decrease plasma theophylline concentrations, as these may affect efficacy and safety. Drug interactions are particularly important and are listed in Table 2. Plasma theophylline concentrations

should be checked if there are any adverse effects or if there are concerns about compliance. Theophylline should never be given with roflumilast, as both inhibit PDE4.

GUIDELINES

The Global Initiative for Asthma recommends that theophylline should be considered as additional treatment when asthma is not controlled on inhaled corticosteroids (step 3) but is less preferred than a LABA and may be added for patients not controlled on inhaled corticosteroids and LABA (steps 4 and 5) (51). In children, low-dose theophylline may be used as a controller at step 2 but is less effective than inhaled corticosteroids. Intravenous aminophylline is not recommended for acute severe asthma.

The Global Initiative for Chronic Obstructive Lung Disease recommends that theophylline be used as a bronchodilator only if inhaled long-acting bronchodilators are unavailable or unaffordable (52). Intravenous aminophylline is not recommended for acute exacerbations.

FUTURE DEVELOPMENTS

Theophylline is a relatively poor bronchodilator, as adverse effects limit the dose and make it less effective than inhaled bronchodilators. However, there is interest in exploring its antiinflammatory effects and its potential to reverse corticosteroid resistance at lower doses that would largely avoid side effects. Low concentrations of theophylline restore reduced HDAC2 to normal and therefore may reverse corticosteroid resistance in COPD and in severe asthma and in smokers with asthma. This effect is achieved by inhibition of phosphoinositidePI3Kδ (13), suggesting that PI3Kδ inhibitors may be developed in the future to treat corticosteroid-resistant airway obstruction. Theophylline is also a nonselective inhibitor of PDE isoenzymes, which may account for the effects of theophylline at higher doses. PDE4 inhibition may mediate antiinflammatory effects but also the common side effects. The selective PDE4 inhibitor roflumilast is now marketed in several countries as an antiinflammatory treatment for COPD, but its clinical efficacy is limited by side effects such as diarrhea, nausea, and headaches, which also occur with high doses of theophylline (53). Other PDE4 inhibitors are in clinical development, although several have failed, including inhaled PDE4 inhibitors. An inhaled PDE3/4 inhibitor, which has a bronchodilator effect due to PDE3 inhibition, is also in clinical development (54). However, PDE inhibitors do not have any effect on HDAC2 so have no potential to reverse corticosteroid resistance. Currently, clinical trials are in progress to assess the potential of low-dose theophylline to reverse corticosteroid resistance in COPD.

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Weinberger M, Hendeles L. Theophylline in asthma. *N Engl J Med* 1996; 334:1380–1388.
2. Rabe KF, Magnussen H, Dent G. Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. *Eur Respir J* 1995;8:637–642.
3. Dent G, Giembycz MA, Rabe KF, Wolf B, Barnes PJ, Magnussen H. Theophylline suppresses human alveolar macrophage respiratory burst through phosphodiesterase inhibition. *Am J Respir Cell Mol Biol* 1994; 10:565–572.
4. Polosa R, Blackburn MR. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol Sci* 2009;30:528–535.
5. Mascali JJ, Cvietusa P, Negri J, Borish L. Anti-inflammatory effects of theophylline: modulation of cytokine production. *Ann Allergy Asthma Immunol* 1996;77:34–38.
6. Oliver B, Tomita K, Keller A, Caramori G, Adcock I, Chung KF, Barnes PJ, Lim S. Low-dose theophylline does not exert its anti-inflammatory effects in mild asthma through upregulation of interleukin-10 in alveolar macrophages. *Allergy* 2001;56:1087–1090.
7. Ichiyama T, Hasegawa S, Matsubara T, Hayashi T, Furukawa S. Theophylline inhibits NF-κ B activation and I κ B α degradation in human pulmonary epithelial cells. *Naunyn Schmiedebergs Arch Pharmacol* 2001;364:558–561.
8. Chung IY, Nam-Kung EK, Lee NM, Chang HS, Kim DJ, Kim YH, Park CS. The downregulation of Bcl-2 expression is necessary for theophylline-induced apoptosis of eosinophil. *Cell Immunol* 2000;203:95–102.
9. Ohta K, Yamashita N. Apoptosis of eosinophils and lymphocytes in allergic inflammation. *J Allergy Clin Immunol* 1999;104:14–21.
10. Moonen HJ, Geraets L, Vaarhorst A, Bast A, Wouters EF, Hageman GJ. Theophylline prevents NAD+ depletion via PARP-1 inhibition in human pulmonary epithelial cells. *Biochem Biophys Res Commun* 2005;338:1805–1810.
11. Ito K, Lim S, Caramori G, Cosio B, Chung KF, Adcock IM, Barnes PJ. A molecular mechanism of action of theophylline: Induction of histone deacetylase activity to decrease inflammatory gene expression. *Proc Natl Acad Sci USA* 2002;99:8921–8926.
12. Cosio BG, Tsaprouni L, Ito K, Jazrawi E, Adcock IM, Barnes PJ. Theophylline restores histone deacetylase activity and steroid responses in COPD macrophages. *J Exp Med* 2004;200:689–695.
13. To Y, Ito K, Kizawa Y, Failla M, Ito M, Kusama T, Elliot M, Hogg JC, Adcock IM, Barnes PJ. Targeting phosphoinositide-3-kinase-δ with theophylline reverses corticosteroid insensitivity in COPD. *Am J Respir Crit Care Med* 2010;182:897–904.
14. Osoata GO, Yamamura S, Ito M, Vuppusetty C, Adcock IM, Barnes PJ, Ito K. Nitration of distinct tyrosine residues causes inactivation of histone deacetylase 2. *Biochem Biophys Res Commun* 2009;384:366–371.
15. Hirano T, Yamagata T, Gohda M, Yamagata Y, Ichikawa T, Yanagisawa S, Ueshima K, Akamatsu K, Nakanishi M, Matsunaga K, *et al.* Inhibition of reactive nitrogen species production in COPD airways: comparison of inhaled corticosteroid and oral theophylline. *Thorax* 2006;61:761–766.
16. Zhang ZY, Kaminsky LS. Characterization of human cytochromes P450 involved in theophylline 8-hydroxylation. *Biochem Pharmacol* 1995; 50:205–211.
17. Nahata M. Drug interactions with azithromycin and the macrolides: an overview. *J Antimicrob Chemother* 1996;37:133–142.
18. Chrystyn H, Mulley BA, Peake MD. Dose response relation to oral theophylline in severe chronic obstructive airways disease. *BMJ* 1988;297:1506–1510.
19. Barnes PJ. Theophylline: new perspectives for an old drug. *Am J Respir Crit Care Med* 2003;167:813–818.
20. Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet* 1994;343:1006–1008.
21. Lim S, Tomita K, Caramori G, Jatakanon A, Oliver B, Keller A, Adcock I, Chung KF, Barnes PJ. Low-dose theophylline reduces eosinophilic inflammation but not exhaled nitric oxide in mild asthma. *Am J Respir Crit Care Med* 2001;164:273–276.
22. Kraft M, Torvik JA, Trudeau JB, Wenzel SE, Martin RJ. Theophylline: potential antiinflammatory effects in nocturnal asthma. *J Allergy Clin Immunol* 1996;97:1242–1246.
23. Culpitt SV, de Matos C, Russell RE, Donnelly LE, Rogers DF, Barnes PJ. Effect of theophylline on induced sputum inflammatory indices and neutrophil chemotaxis in COPD. *Am J Respir Crit Care Med* 2002;165:1371–1376.
24. Kobayashi M, Nasuhara Y, Betsuyaku T, Shibuya E, Tanino Y, Tanino M, Takamura K, Nagai K, Hosokawa T, Nishimura M. Effect of low-dose theophylline on airway inflammation in COPD. *Respirology* 2004;9:249–254.
25. Kanehara M, Yokoyama A, Tomoda Y, Shiota N, Iwamoto H, Ishikawa N, Taooka Y, Haruta Y, Hattori N, Kohno N. Anti-inflammatory effects and clinical efficacy of theophylline and tulobuterol in mild-to-moderate chronic obstructive pulmonary disease. *Pulm Pharmacol Ther* 2008;21:874–878.
26. Hidi R, Timmermans S, Liu E, Schudt C, Dent G, Holgate ST, Djukanović R. Phosphodiesterase and cyclic adenosine monophosphate-dependent inhibition of T-lymphocyte chemotaxis. *Eur Respir J* 2000;15:342–349.

27. Kidney J, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma. Demonstration by withdrawal of therapy. *Am J Respir Crit Care Med* 1995;151:1907–1914.
28. Aubier M, De Troyer A, Sampson M, Macklem PT, Roussos Ch. Aminophylline improves diaphragmatic contractility. *N Engl J Med* 1981;305:249–252.
29. Moxham J. Aminophylline and the respiratory muscles: an alternative view. *Clin Chest Med* 1988;9:325–336.
30. Nair P, Milan SJ, Rowe BH. Addition of intravenous aminophylline to inhaled beta(2)-agonists in adults with acute asthma. *Cochrane Database Syst Rev* 2012;12:CD002742.
31. Mitra A, Bassler D, Goodman K, Lasserson TJ, Ducharme FM. Intravenous aminophylline for acute severe asthma in children over two years receiving inhaled bronchodilators. *Cochrane Database Syst Rev* 2005;CD001276.
32. Duffy N, Walker P, Diamantea F, Calverley PM, Davies L. Intravenous aminophylline in patients admitted to hospital with non-acidotic exacerbations of chronic obstructive pulmonary disease: a prospective randomised controlled trial. *Thorax* 2005;60:713–717.
33. Barr RG, Rowe BH, Camargo CA Jr. Methylxanthines for exacerbations of chronic obstructive pulmonary disease: meta-analysis of randomised trials. *BMJ* 2003;327:643.
34. Wilson AJ, Gibson PG, Coughlan J. Long acting beta-agonists versus theophylline for maintenance treatment of asthma. *Cochrane Database Syst Rev* 2000;2:CD001281.
35. Brenner MR, Berkowitz R, Marshall N, Strunk RC. Need for theophylline in severe steroid-requiring asthmatics. *Clin Allergy* 1988;18:143–150.
36. Rivington RN, Boulet LP, Cote J, Kreisman H, Small DI, Alexander M, Day A, Harsanyi Z, Darke AC. Efficacy of slow-release theophylline, inhaled salbutamol and their combination in asthmatic patients on high-dose inhaled steroids. *Am J Respir Crit Care Med* 1995;151:325–332.
37. Evans DJ, Taylor DA, Zetterstrom O, Chung KF, O'Connor BJ, Barnes PJ. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. *N Engl J Med* 1997;337:1412–1418.
38. Ukena D, Harnest U, Sakalauskas R, Magyar P, Vetter N, Steffen H, Leichtl S, Rathgeb F, Keller A, Steinijans VW. Comparison of addition of theophylline to inhaled steroid with doubling of the dose of inhaled steroid in asthma. *Eur Respir J* 1997;10:2754–2760.
39. Lim S, Groneberg D, Fischer A, Oates T, Caramori G, Mattos W, Adcock I, Barnes PJ, Chung KF. Expression of heme oxygenase isoenzymes 1 and 2 in normal and asthmatic airways: effect of inhaled corticosteroids. *Am J Respir Crit Care Med* 2000;162:1912–1918.
40. Spears M, Donnelly I, Jolly L, Brannigan M, Ito K, McSharry C, Lafferty J, Chaudhuri R, Braganza G, Adcock IM, *et al*. Effect of low-dose theophylline plus beclometasone on lung function in smokers with asthma: a pilot study. *Eur Respir J* 2009;33:1010–1017.
41. Marwick JA, Wallis G, Meja K, Kuster B, Bouwmeester T, Chakravarty P, Fletcher D, Whittaker PA, Barnes PJ, Ito K, *et al*. Oxidative stress modulates theophylline effects on steroid responsiveness. *Biochem Biophys Res Commun* 2008;377:797–802.
42. Murciano D, Auclair MH, Pariente R, Aubier M. A randomized, controlled trial of theophylline in patients with severe chronic obstructive pulmonary disease. *N Engl J Med* 1989;320:1521–1525.
43. ZuWallack RL, Mahler DA, Reilly D, Church N, Emmett A, Rickard K, Knobil K. Salmeterol plus theophylline combination therapy in the treatment of COPD. *Chest* 2001;119:1661–1670.
44. Zhou Y, Wang X, Zeng X, Qiu R, Xie J, Liu S, Zheng J, Zhong N, Ran P. Positive benefits of theophylline in a randomized, double-blind, parallel-group, placebo-controlled study of low-dose, slow-release theophylline in the treatment of COPD for 1 year. *Respirology* 2006;11:603–610.
45. Cosio BG, Iglesias A, Rios A, Noguera A, Sala E, Ito K, Barnes PJ, Agusti A. Low-dose theophylline enhances the anti-inflammatory effects of steroids during exacerbations of chronic obstructive pulmonary disease. *Thorax* 2009;64:424–429.
46. Ford PA, Durham AL, Russell REK, Gordon F, Adcock IM, Barnes PJ. Treatment effects of low-dose theophylline combined with an inhaled corticosteroid in COPD. *Chest* 2010;137:1338–1344.
47. Henderson-Smart DJ, De Paoli AG. Methylxanthine treatment for apnoea in preterm infants. *Cochrane Database Syst Rev* 2010;12:CD000140.
48. Cushley MJ, Holgate ST. Bronchodilator actions of xanthine derivatives administered by inhalation in asthma. *Thorax* 1985;40:176–179.
49. Voduc N, Alvarez GG, Amjadi K, Tessier C, Sabri E, Aaron SD. Effect of theophylline on exercise capacity in COPD patients treated with combination long-acting bronchodilator therapy: a pilot study. *Int J Chron Obstruct Pulmon Dis* 2012;7:245–252.
50. Shukla D, Chakraborty S, Singh S, Mishra B. Doxofylline: a promising methylxanthine derivative for the treatment of asthma and chronic obstructive pulmonary disease. *Expert Opin Pharmacother* 2009;10:2343–2356.
51. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, *et al*. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;31:143–178.
52. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013;187:347–365.
53. Michalski JM, Golden G, Ikari J, Rennard SI. PDE4: a novel target in the treatment of chronic obstructive pulmonary disease. *Clin Pharmacol Ther* 2012;91:134–142.
54. Banner KH, Press NJ. Dual PDE3/4 inhibitors as therapeutic agents for chronic obstructive pulmonary disease. *Br J Pharmacol* 2009;157:892–906.