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COMMENTARY

Upping the antedrug: is a novel anti-inflammatory Toll-like receptor 7 agonist also a bronchodilator?

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In this issue of *British Journal of Pharmacology*, Biffen and colleagues present a novel Toll-like receptor 7 (TLR7) antedrug to treat allergic disease that is rapidly metabolized in the lung to limit side effects due to systemic exposure. Asthma is characterized as an allergic disease of the lung, and TLR7 agonists are proposed to ameliorate allergic inflammation in the lung, a characteristic of prophylactic medications. We have previously shown that TLR7 agonists of multiple structural classes are acute bronchodilators, characteristic of rescue medication for asthma attacks. It will be interesting to determine whether the bronchodilating effect extends to the novel class of TLR7 agonists described here for a prophylactic and rescue therapy in one drug. Combined with the antedrug approach, this would further limit side effects improving on current combination therapies.

LINKED ARTICLE
This article is a commentary on Biffen *et al.*, pp. 573–586 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01790.x

Abbreviations
BK/MaxiK, large-conductance calcium and voltage-gated potassium channel; R837, R848, imidazoquinoline TLR7 agonists; ssRNA, single-stranded RNA; Th1, Type-1 T-helper; Th2, Type-2 T-helper; TLR7, Toll-like receptor 7

Toll-like receptor 7 (TLR7) responds to single-stranded RNA (ssRNA) and provides a mechanism for cells to respond rapidly to viral infection. Synthetic TLR7 agonists are potent immunomodulators with substantial potential for treatment of airway diseases. However, systemic exposure may produce side effects ranging from flu-like symptoms to sepsis (Pockros *et al.*, 2007; Fidock *et al.* 2011).

The work published by Biffen *et al.* (2012) in this issue of *British Journal of Pharmacology* highlights a novel TLR7 agonist class to combat allergic inflammation in the lung, while limiting systemic exposure and associated side effects. This was accomplished by synthesizing an antedrug ester of an adenine derivative that is a TLR7 agonist with no human TLR8 activity. Upon exposure to butyrylcholinesterase in the plasma, the antedrug is cleaved to its acid metabolite that is significantly less potent at TLR7. In this way, the drug is active at TLR7 in the lung after intranasal administration and pulmonary aspiration, but systemic exposure is very limited. This antedrug approach is an elegant solution to a large barrier to clinical use. The briefer activation of TLR7 due to the antedrug's rapid metabolism also results in preferential activation of interferon production over NF-κB-regulated genes, another mechanism by which side effects might be limited. This exciting new evidence of selective regulation of the complex signal cascades activated by TLRs at the level of receptor occupancy suggests further refinement of drug profiles based on their on- and off-rate kinetics.

As this is part of a new structural class of TLR7 agonists, this work is important for both therapeutic and research purposes. Currently, synthetic TLR7 agonists are mostly limited to imidazoquinolines (Hemmi *et al.*, 2002), guanine nucleoside analogues (Lee *et al.*, 2003) and ssRNA oligonucleotides (Diebold *et al.*, 2004; Diebold *et al.*, 2004). Although 8-oxoadenine derivatives, the structural class of the antedrug described here, have more recently been reported as TLR7 agonists (Lee *et al.*, 2006), the field continues to be

Table 1

Studies of chronic administration of TLR7 agonists for airway inflammation

TLR7 agonist	Species/model	Airway inflammation	Airway hyperreactivity	Author, year
AZ12441970	Mouse/ovalbumin-sensitization	↑IFNs, ↓Th2 cytokines, eosinophils	Not tested	(Biffen *et al.*, 2012)
Substitute adenine-2	Mouse/ovalbumin-sensitization	↑Th1 cytokines ↓Th2 cytokines, eosinophils, goblet cells, mucus, IgE	Not tested	(Vultaggio *et al.*, 2009)
R-848	Mouse/ovalbumin-sensitization	↑Th1 cytokines ↓Th2 cytokines, eosinophils, mucus gland hyperplasia	↓	(Quarcoo *et al.*, 2004)
R-848	Mouse/ovalbumin-sensitization	↓Th2 cytokines, eosinophils, IgE	↓	(Moisan *et al.*, 2006)
R-848	Mouse/ovalbumin-sensitization	↓Th2 cytokines, eosinophils, goblet cells	↓	(Sel *et al.*, 2007)
R-848	Rat/ovalbumin-sensitization	↓Th2 cytokines, Th1 cytokines, eosinophils, airway remodelling	Not tested	(Camateros *et al.*, 2007)
R-848	Mouse/ovalbumin-sensitization	↑NK cells ↓eosinophils, adhesion molecules, chemokines, airway remodelling	Not tested	(Camateros *et al.*, 2009)
R-848	Mouse/ovalbumin-sensitization	↑IFNs, ↓Th2 cytokines, eosinophils. goblet cells, mucus	↓	(Xirakia *et al.*, 2010)
R-848	Mouse/ovalbumin-sensitization	↓Th2 cytokines, eosinophils	↓	(Duechs *et al.*, 2011)
R-848	Mouse/ovalbumin-sensitization	↓regulatory T cells, IFN ↓Th2 cytokines, eotaxin, eosinophils	↓	(Van *et al.*, 2011)
R837	Rat/virus-infection	↓eosinophils, virus titres	↓	(Stokes *et al.*, 1998)
R837	Mouse/ovalbumin-sensitization	↓Th2 chemokines, eosinophils, macrophages, lymphocytes	↓	(Jin *et al.*, 2006)
R837	Rat/ovalbumin-sensitization	↑Th1 cytokines, T-bet ↓Th2 cytokines, GATA-3, eosinophils, airway remodelling	↓	(Bian *et al.*, 2006)
R837, R848	Guinea-pig/no asthma model	Not tested	↓Smooth muscle responsiveness	(Ekman *et al.*, 2010)
R837	Rat/ovalbumin-sensitization	↑IFNs ↓Th2 cytokines, IgE, lymphocytes	Not tested	(Meng *et al.*, 2011)

dominated by work with the imidazoquinolines, such as imiquimod (R837) and resiquimod (R848). Off-target effects of imidazoquinoline TLR7 agonists include antagonism of adenosine receptors (Schon *et al.*, 2006) and PDE inhibition (Seiler *et al.*, 1991), as well as activation of the highly homologous TLR8 (Diebold, 2008). This has added to the challenge of defining the effects of TLR7 stimulation with currently available pharmacological tools. The discovery of a high-potency TLR7-specific antedrug agonist is beneficial to the field for therapeutic potential with decreased side effects, but it is also important for distinguishing effects of TLR7 agonism from off-target effects in general research.

The immunomodulatory potential of TLR7 agonists has been investigated to remedy allergic diseases, such as asthma, by reversing type-2 T-helper (Th2) inflammation and promoting type-1 T-helper (Th1) inflammation. Similar to the data presented by Biffen *et al.* (2012), these drugs have been shown to reverse airway inflammation in a number of species and models (Table 1). Additionally, some of these groups have demonstrated reversal of the airway hyperreactivity characteristic of asthma. It will be important to determine if TLR7 agonists of the 8-oxoadenine structural class prevent airway hyperreactivity in addition to the anti-inflammatory properties reported here.

Airway hyperreactivity is a consequence of inflammation. This has led to a conceptualization of asthma medications as divided into anti-inflammatory drugs (primarily glucocorticoids) and bronchodilators (primarily β agonists). In this

framework, anti-inflammatory drugs are useful as chronic prophylactic therapies, but a rapid bronchodilator for rescue from acute bronchoconstriction is usually also necessary either as co-prescription or co-formulation.

We have previously demonstrated that TLR7 agonists are rapid bronchodilators, reducing bronchoconstriction within minutes of i.v. administration to guinea-pigs *in vivo* (Kaufman *et al.*, 2011). The relaxant effect is also observed against contraction of the guinea-pig isolated trachea *in vitro* for both imidazoquinoline and ssRNA structural classes of TLR7 agonists tested. This rapid bronchodilating effect is mediated at the airway smooth muscle by a TLR7-dependent pathway and a TLR7-independent pathway, as only part of the effect can be reversed by a TLR7 antagonist, IRS661. The TLR7-dependent pathway is mediated by NO, whereas the independent component is mediated by prostaglandins and the large conductance calcium and voltage-gated potassium channel (BK/MaxiK). Relaxant effects of TLR7 agonists extend to human and mouse isolated tracheas *in vitro* (unpubl. data). Based on available ligand selectivity information in the human, the TLR7-independent component of bronchodilatation is probably mediated by the highly homologous TLR8. We propose a protective mechanism conserved across three distantly related mammalian species by which the airways dilate during a respiratory virus infection to accommodate the passage of air during the increased airway obstruction associated with inflammation necessary to clear a virus infection. This type of protective mechanism has also been described for other pathogen-associated molecular patterns such as bacterial ligands for bitter taste receptors expressed in the airways (Deshpande *et al.*, 2010).

The bronchodilating effect we describe is very different from the inhibition of airway hyperreactivity reported by others. The reports are from chronic models of administration of the TLR7 agonists at the time of allergen sensitization or challenge, days before airway physiology measurements. In these reports, bronchoconstriction is not inhibited but is rather returned from hyperreactive to control values, likely due to the reversal of Th2-type inflammation. In contrast, we describe acute inhibition of bronchoconstriction within minutes of TLR7 agonist administration. This is inhibition of bronchoconstriction, the normal physiological response to vagal stimulation or ACh action at airway smooth muscle. This rapid bronchodilating effect is a desirable characteristic of rescue medication for active bronchoconstriction. That the bronchodilating effect translates to human tissue suggests TLR7 agonists could be used successfully for this therapeutic purpose. The rapid time frame of the bronchodilating effect also emphasizes the need to consider rapid effects independent of longer-term changes in gene expression associated with canonical TLR7 signalling.

Combined with the anti-Th2-inflammatory effects of chronic TLR7 agonist administration, the rapid bronchodilating effect of these drugs suggests they might serve as both rescue bronchodilators and prophylactic anti-inflammatories. These properties combined into one medication are desirable as currently available combination therapies to achieve both rescue and prophylaxis can be associated with increased side effects. The addition of the antedrug concept to limit systemic exposure would further limit side effects of a single drug for prophylactic and rescue therapy. It will be interesting to determine if the rapid bronchodilating properties of imidazoquinoline and ssRNA TLR7 ligands extends to the novel class of TLR7 agonists described by Biffen and colleagues.

Conflicts of interest

The authors have no conflicts to disclose at this time.

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